Remarks

Summary of this Response to the Office Action of 9/22/08

Claims 91 to 166 (a total of 76 pending claims) were previously pending in the application; claims 140-166 were withdrawn from consideration. In the non-final Office Action of 9/22/2008 claims 91-139 were rejected. The applicants have responded by amending claims 91-93, 104-105, 108 and 109, and canceling claims 94-103, 106-107, and 110-166. New claims 167-235 have been added for a total of 76 pending claims, unchanged from the previous total.

The applicants have addressed the Examiner statement of lack of priority related to the claim limitation "thousands of bi-allelic markers" and the indefiniteness rejection also related to the claim limitation "thousands of bi-allelic markers" **(see pp. 15-24).** An act or process type limitation has been added to independent claim 91 **(see claim 91 and p. 52).** In addition, several "whereby clauses" that are not true limitations are now effectively included in all claims to help to more clearly delineate the "metes and bounds" of the claimed invention(s) **(see the claims and pp. 17-18).**

Remarks regarding the proper construction of claims that include the limitation "thousands of bi-allelic markers" are respectfully given **(see pp. 15-16, 19-20).** These Arguments and Remarks indicate that the pending claims are indeed entitled to the priority of earlier filed parent and priority applications and that the references McGinnis (1999) and Cohen (1999) are not "prior art" against the pending claims **(see pp. 20-24).**

A rebuttal of the Examiner statement of no evidence of unexpected results is given. A great deal of objective evidence of the achievement of "unexpected results" by the claimed invention(s) is presented **(pp. 24-41 & Summary pp. 40-41).** A further rebuttal of the obviousness rejection in the recent Office Action is then also made. Specifically

a rebuttal of the case of prima facie obviousness based on the Cohen (1997) and Kruglyak (1995) references is respectfully submitted **(see pp. 41-51).** Cohen (1997) and Kruglyak (1995) as whole references lead away from the claimed invention(s). A minor allele frequency subrange ("0.3 to 0.5" or "not less than about 0.3") described in Cohen (1997) that "touches" or "barely overlaps" a subrange ("less than or equal to 0.3") present in some claims does not by itself establish obviousness.

Similar claims are generally grouped together. Claims 93-211 deal with allele specific oligonucleotides complementary to SNPs, of these claims 201-211 deal with apparatus. Claims 212-235 deal with oligonucleotides useful as PCR primers with SNPs, e.g., for genotyping. **Support for limitations in the presently pending claims** to ensure compliance with the Written Description Requirement of 35 U.S.C. 112 is cited in the text of the present application **(see pp. 51-70).** Such support in the PCT parent, PCT/US99/04376 and in Provisional priority application 60/076102 is also cited in order to ensure that later filed references (such as McGinnis (1999) and Cohen (1999)) are not cited as "prior art" against the presently pending claims.

Some further Remarks regarding interpretation of the pending claims, claim terminology and claim construction are made; these Remarks do deal with product and product by process type claims. These Remarks include important comments about the act or process type limitations in independent claim 91, claim construction of the pending claims, and claim construction of product by process claims **(see pp. 71-74). Exhibit A, evidence of unobviousness, starts on p. 78. End of Summary of Remarks.**

More Detailed Remarks

The claim limitation "thousands of bi-allelic markers" and the issue of Priority & the Indefiniteness Rejection

Regarding the Examiner's point with respect to Priority (p. 2) in the Office Action of 9/22/2008, the Examiner states: *"Application No. 09/947, 768, fails to provide*

adequate support for claim 91, which requires oligonucleotides complimentary to a group of covering markers, wherein the group comprises thousands of bi-allelic markers with least common allele frequencies less than or equal to 0.3." The applicants respectfully submit that this Examiner's statement is due to an Examiner misconstruction of former claim 91; <u>this is not the claim construction the applicants intended</u>. More specifically the claim requires only that the group of covering markers comprises thousands of covering markers and <u>some</u> (not necessarily thousands) of covering markers have least common allele frequencies less than or equal to 0.3. **The applicants respectfully submit that this construction (<u>some,</u> not necessarily thousands) is in agreement with the plain reading of former claim 91.** The applicants respectfully submit that indeed Application No. 09/947, 768 does support the above construction (<u>some,</u> not necessarily thousands), as the applicants will shortly show below.

<u>Indefiniteness Rejection and the limitation "thousands of bi-allelic markers"</u>

The Examiner also rejected claims 91-139 under 35 USC 112, second paragraph for indefiniteness (pp. 3-4). The Examiner states (emboldening & underlining added) that: *"a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite…..In the present instance, claim 91 recites the broad limitation 'two or more' and the claim also recites **'<u>thousands</u>'** which is the narrower statement of the range/limitation."* The Examiner also refers to MPEP 2173.05(c). MPEP 2173.05(c) states (underlining added): *"Use of a narrow numerical range that falls within a broader range in the same claim may render the claim indefinite when the boundaries of the claim are not discernible. Description of examples and preferences is properly set forth in the specification rather than in a single claim. <u>A narrower range or preferred embodiment may also be set forth in another independent claim or in a dependent claim.</u>"*

In order to overcome this indefiniteness rejection, the applicants have (as suggested by MPEP 2173.05(c)) amended independent claim 91 by deleting the limitation *"wherein the group of covering markers comprises thousands of bi-allelic markers."* And a similar limitation regarding *"thousands of bi-allelic markers"* has been added to

several other dependent claims. Claim 91 has also been narrowed by adding several other limitations.

The CL-F region in claim 91 that is N-covered is now stipulated to be a segment-subrange (a rectangular region that is bounded by a least common allele frequency subrange and by a chromosomal segment). The segment-subrange includes lower frequency points, specifically the subrange of the segment-subrange includes the least common (or minor) allele frequency 0.1. And the segment of the segment-subrange is greater than or equal to the length of the shortest human chromosome, human chromosome 21. Human chromosome 21 is at or about 47 million base pairs in length.

Because of this limitation and other added limitations, claim 91 now includes a “whereby clause” that delineates an approximate minimum number of covering markers with least common (minor) allele frequencies that are less than or equal to 0.3. That minimum number is at least about 24 covering markers that are distributed within the segment of the segment-subrange (the CL-F region) with a minimum density of at least about 1 marker every two million base pairs. This whereby clause is not a true limitation *“because a whereby clause that merely states the result of the limitations in the claim adds nothing to the substance of the claim.”* Texas Instruments Inc. v. United States Int'l Trade Comm'n, 988 F.2d 1165, 1172, 26 USPQ2d 1018, 1023-24 (Fed. Cir. 1993); quoted in Lockheed Martin vs. Space Systems/Loral, 249 F.3d 1314.

There are other similar “whereby clauses” in other dependent claims delineating an increased minimum number of covering markers (e.g., about 24, 96, 288 and 1037 markers) with lower least common (minor) allele frequencies; such as minor allele frequencies less than or equal to 0.3, 0.2 or 0.1. The covering markers delineated in these “whereby clauses” are also distributed within the segment of the segment-subrange (the CL-F region) with an approximate minimum density. **Though these whereby clauses are not true limitations, it is the applicants intention that these**

clauses will help to delineate "the metes and bounds" of the claimed invention(s).

Some examples of these whereby clauses are: (1) *"whereby there are at least about 24 covering markers with least common allele frequencies less than or equal to 0.3 that are distributed within the segment with a density of at least about 1 marker every two million base pairs,"* (2) *"whereby there are at least about 24 covering markers with least common allele frequencies less than or equal to 0.1 that are distributed within the segment with a density of at least about 1 marker every two million base pairs,"* (3)*"whereby there are at least about 96 covering markers with least common allele frequencies less than or equal to 0.2 that are distributed within the segment with a density of at least about 1 marker every five hundred thousand base pairs,"* (4) *"whereby there are at least about 288 covering markers with least common allele frequencies less than or equal to 0.2 that are distributed within the segment with a density of at least about 1 marker every 167 thousand base pairs"* and (5) *"whereby there are at least about 288 covering markers with least common allele frequencies less than or equal to 0.1 that are distributed within the segment with a density of at least about 1 marker every 167 thousand base pairs."*

These whereby clauses delineate groups or subsets (or subgroups) of lower minor allele frequency (lower heterozygosity) covering markers that have the <u>unexpected, unobvious</u> property of increased power to detect linkage in association studies. More specifically the increased power is to detect linkage (in association studies) to lower frequency trait-causing polymorphism alleles, such as a disease allele with a low allele frequency "p," p = 0.1. **The applicants will cite the abundance of evidence for this <u>unexpected, unobvious</u> property of increased power below.**

Because claim 91 now delineates an increased number of covering markers with minor allele frequencies that are less than or equal to 0.3 (rather than simply <u>some,</u>

not necessarily thousands), as was the proper claim construction for former claim 91 noted above, new claim 91 is narrowed with respect to this limitation.

Proper claim construction of the pending claims that include the limitation *"thousands of bi-allelic markers."*

The applicants will now also respectfully submit comments to the Examiner on the proper claim construction for other pending dependent claims that now include the limitation *"thousands of bi-allelic markers."* It is the applicants' respectful intent that these comments will help avoid an Examiner misconstruction of these claims, as the applicants respectfully submit occurred in the previous Office Action of 9/22/08.

A marker being a "covering marker" does not necessarily mean the covering marker actually covers the CL-F region, i.e., that the covering marker is within the covering distance of a CL-F point in the region. In fact it is possible for a CL-F region to actually be covered by only a proper subset (less than all) of the covering markers. This fact is suggested in the present application on p. 10 lines 28-31 and in the PCT parent, PCT/US99/04376, on p. 9 lines 28-31. This fact is also clear, for example, from the definition of systematic covering (present application 10/03718 p. 14 lines 20-25) and definition of N-covering of a CL-F region (present application 10/03718 p. 13 lines 34-36, p. 14 lines 26-28 and parent PCT/US99/04376 p. 12 lines 34-36, lines 26-28).

More specifically in the case of N-covering of a CL-F region *"each point in the* [CL-F] *region is N covered,"* see p. 14 lines 26-28. This means that any one point (each point) in the CL-F region is within the two-dimensional covering distance (δ or [x, y]) of *"each of N or more of the covering markers,"* see p. 13 lines 34-36. So, for example, if the CL-F region is a single point (as is possible, see p. 10 lines 26-27), it is only necessary for each of N or more of the covering markers to be within the covering distance (δ or [x, y]) of the single point. To push the example further, if the CL-F region is a single point, there are *"thousands of bi-allelic covering markers,"* and N=1, it is only

necessary for one covering marker of the thousands of covering markers to be within the covering distance (δ or [x, y]) of the single point in the CL-F region. **This simple example illustrates, as stated above, that it is possible for a CL-F region to actually be covered by only a proper subset (less than all, even much less than all) of the covering markers.**

In addition to help clarify this point further and the "metes and bounds" of the claimed invention(s), claim 91 has been amended to include the whereby clause *"whereby each point in the region is within the distance [x,y] of each of N or more of the covering markers."* This whereby clause is not a true limitation, it follows from the other limitations in the claim and the definition of N-covering cited above (p. 13 lines 34-36 & p. 14 lines 26-28).

<u>**The presently pending claims (with the proper construction) are in fact supported by parent and priority applications; therefore references McGinnis (1999) and Cohen (1999) are not "prior art" against the pending claims, as the applicants will now show.**</u>

In the Office Action 9/22/08, under ***Priority*** on p. 2, the Examiner states: *"…prior-filed application, Application No. 09/947,768, fails to provide adequate support for claim 91, which requires oligonucleotides complimentary* [sic] *to a group of covering markers, wherein the group comprises thousands of bi-allelic markers with least common allele frequencies less than or equal to 0.3."* As the applicants have respectfully stated above, this is not what the applicants intended to claim in former claim 91, but is a misconstruction of former claim 91.

Similarly, the presently pending claims, such as dependent claims that now include the limitation *"thousands of bi-allelic markers"*, are <u>not</u> claiming *"a group of covering markers, wherein the group comprises thousands of bi-allelic markers with least common allele frequencies less than or equal to 0.3"* as the Examiner states in the

Office Action. Rather it is only necessary that <u>some</u>, not necessarily thousands, of the covering markers have least common allele frequencies less than or equal to 0.3.

The applicants respectfully submit that indeed priority parent application 09/947,768, parent PCT/US99/04376 and other priority applications (such as Provisional 60/076102) support this (proper) construction of the claim(s). More specifically the clause *"thousands of bi-allelic markers"* appears in 09/947,768 in connection with a way to implement the new Two-Dimensional Linkage Study Techniques of the present application (see for example p. 25 lines 11-12, p. 36 lines 5-8 of 09/947,768, and p. 49 lines 1-16). In addition, parent application PCT/US99/04376 is incorporated by reference into 09/947,768, see p. 68 line 34. The clause *"thousands of bi-allelic markers"* also appears in parent PCT/US99/04376 in connection with a way to implement the new Two-Dimensional Linkage Study Techniques of the present application (see PCT/US99/04376 for example p. 24 lines 1-2, p. 34 line 6 and p. 47 lines 1 to 16). And as stated above parent application PCT/US99/04376 is incorporated by reference into the present application, see p. 1, lines 8-11. In addition, the clause *"thousands of bi-allelic markers"* also appears in the present application in connection with a way to implement the new Two-Dimensional Linkage Study Techniques of the present application (see for example p. 25 lines 9-10, p. 35 lines 8-11 and p. 48 lines 1-16 of the present application).

The applicants respectfully submit that the presently pending claims with the clause *"thousands of bi-allelic markers"* with the intended construction above are indeed supported in accordance with the Written Description Requirement (MPEP 2163). The intended construction is that it is only necessary that <u>some</u>, not necessarily thousands, of the covering markers have least common allele frequencies less than or equal to 0.3.

MPEP 2163 states in part: *"To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in*

the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., Moba, B.V. v. Diamond Automation, Inc., 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563, 19 USPQ2d at 1116." The Description of the present application (and PCT parent) describes *"thousands of bi-allelic markers"* in connection with a way to implement the described new Two-Dimensional Linkage Study Techniques under Oligonucleotide Technology of the present application using **oligonucleotides that are complementary to the thousands of bi-allelic markers** (see for example p. 25 lines 9-10, p. 34 lines 25-36, p. 35 lines 8-11 and p. 48 lines 1-16 of the present application).

The applicants therefore respectfully submit that the Description of the present application (and PCT parent) support the claims with the limitation *"thousands of bi-allelic markers"* with the intended construction. The applicants respectfully submit that this support is further reinforced **for a person of ordinary skill in the art** by the context in the art at the time of filing. At the time of filing it was known in the art that thousands, even millions, of bi-allelic markers (SNPs) would likely become identified and become available for future use in linkage studies; see for example p. 21 mid right column of Kruglyak (The use of a genetic map of biallelic markers in linkage studies. Nature Genetics, September 1997, vol.17, pp. 21-24) that states *"classic estimates of more than 1 per 1,000 base pairs, or more than 3 million* [SNPs] *in the genome."* It should be noted that 3 million SNPs in the entire genome translates to about 130, 000 SNPs (3 million/23) per human chromosome. This Kruglyak (1997) reference is cited in the Background of the present application, see footnote 4, p. 5. A copy of this Kruglyak (1997) reference was supplied as Reference F in an Information Disclosure Statement (IDS) filed in November 2000 for parent application 09/623,068. **The applicants will also expeditiously supply the Examiner with another copy of this Kruglyak (1997) reference (or any other reference referred to herein) for his convenience if he should so desire.**

The applicants respectfully submit that this support is further reinforced **<u>for a person of ordinary skill in the art</u>** by a reading of the Description that favors higher power embodiments of the invention with smaller values of the covering distance (δ) and higher values of N, including the largest possible value of N (see p. 27 lines 32-39 of the present application and the PCT parent p. 26 lines 23-30). Smaller values of the covering distance (δ) and higher values of N, including up to the largest possible value of N, mean more covering markers. And as stated above, thousands, even millions of such bi-allelic covering markers were estimated to be available for future use in linkage studies (and related techniques).

The applicants therefore respectfully submit that a reading of the Description by a person of ordinary skill in the art (of the present application and the PCT parent) support the claims with the limitation *"thousands of bi-allelic markers"* with the intended construction.

<u>The issue of Priority: McGinnis (1999) and Cohen (1999) are not "prior art" against the pending claims.</u>

The applicants therefore also respectfully submit that later references cited by the Examiner in the rejections of the Office Action of 9/22/08 are not "prior art" against former claim 91 or the presently pending claims. More specifically since the PCT parent supports the presently pending claims, then McGinnis et. al. (PCT/US/99/04376, WO/1999/43858) cited by the Examiner mid page 8 in the Office Action of 9/22/08 is not "prior art" against former claim 91 or the presently pending claims.

Similarly Cohen (EP 0892068; Date of Publication: 20 Jan 1999) cited top p. 5 and mid p. 7 to mid p. 9 of the Office Action of 9/22/08 is also not "prior art" against the presently pending claims. This is because Provisional application 60/076102 (filed 26

Feb 1998) is a priority application for both the present application and the PCT parent. The Description of application 60/076102 also describes *"thousands of bi-allelic markers"* in connection with a way to implement the new Two-Dimensional Linkage Study Techniques under <u>Oligonucleotide Technology</u> of application 60/076102 using <u>**oligonucleotides that are complementary to the thousands of bi-allelic markers**</u> (see for example p. 46 lines 7-15, and p. 53 lines 39-52 of application 60/076102). Similarly application 60/076102 contains text describing higher power embodiments with smaller values of the covering distance (δ) and higher values of N, including up to the largest possible value of N that mean more covering markers (see 60/06102 p. 35 lines 44-49). This text is essentially identical to the corresponding text cited above in the PCT parent and the present application. (More information on 60/06102 and the inventor's unpublished manuscript which was included with, and incorporated by reference into, 60/06102 is given below.) Since provisional application 60/076102 was filed in 1998, before Cohen (1999), the applicants also respectfully submit that Cohen (1999) is also not "prior art" against the presently pending claims.

<u>**Rebuttal of Examiner statement of no objective evidence of unexpected results, and then a further rebuttal of the prima facie case of obviousness**</u>

The applicants will cite support for the limitations added to the presently pending claims (such as those that lead to the "whereby clauses") to ensure compliance with the Written Description Requirement below. The applicants will continue to cite other evidence below that McGinnis (1999) and Cohen (1999) are not "prior art" against the pending claims. The applicants will also make a rebuttal of the prima facie case of obviousness based on the Cohen (1997) and Kruglyak (1995) references below. **However, the applicants will now first address the Examiner statement of no objective evidence of unexpected results in the Office Action of 9/22/2008 (p. 9).**

These "unexpected results" are important because, among other things, they make the prima facie case of obviousness (based on Cohen (1997) & Kruglyak

(1995)) moot. This is because the groups or subsets (or subgroups) of lower minor allele frequency (lower heterozygosity) covering markers delineated by the "whereby clauses" in the pending claims cannot be "at once envisaged" (in light of Cohen (1997) and Kruglyak (1995)) by a person of ordinary skill in the art. **The applicants respectfully submit that unless these groups/subgroups of covering markers (delineated by "whereby clauses") can be "at once envisaged," as required by the guidance of MPEP sections such as 2131.02, 2131.03, 2144.05, and 2144.08 (e.g., 2144.08 II A 4. (a)), then the pending claims are not 102/103 obvious.**

The groups or subgroups of lower minor allele frequency (lower heterozygosity) covering markers delineated by whereby clauses in the presently pending claims have the <u>unexpected, unobvious</u> property of increased power to detect linkage in association studies. More specifically the increased power is to detect linkage (in association studies) to lower frequency trait-causing polymorphism alleles, such as a disease allele with a low allele frequency "p," p = 0.1. **The applicants will now cite the abundance of evidence for this <u>unexpected, unobvious</u> property of increased power below.**

Rebuttal of Obviousness Rejection based on lack of objective evidence of Unexpected Results

A key part of the Examiner's rejection for obviousness is this Examiner statement (see p. 9): *"Applicant's reference to the specification [p.21] does not present any experimental data showing unexpected results. Due to absence of experimental data, tests, or calculations comparing applicant's power studies using bi-allelic markers with those of the closest prior art, applicant's assertion of unexpected results constitute mere argument."*

The applicants respectfully, but strongly disagree with this statement: what the Examiner is saying here is simply factually incorrect. Page 21 of the published application (referred to in the above Examiner quote) corresponds to p. 39 line 15 to p. 42 line 3 of the originally filed application 10/037,718 (and also corresponds to p. 38

line 2 to p. 41 line 3 of parent application PCT/US99/04376, which is part of the present application through incorporation by reference).

Similarly this subject matter is in the inventor's unpublished manuscript. Essentials of p. 21 (such as Equation 2, Table 2 and explanation of their significance the text (p. 42 line 5 to p. 4 line 2) in the present application and (p. 41 line 5 to p. 43 line 2) in the PCT parent entitled: <u>Importance of disequilibrium and marker heterozygosity (i.e. marker allele frequency) in detecting linkage</u> are also part of Provisional priority application 60/076102 filed 26 Feb 1998. These essentials (Equation 2, Table 2, and text entitled: <u>Importance of disequilibrium and marker heterozygosity (i.e. marker allele frequency) in detecting linkage)</u> are in the inventor's unpublished manuscript that was included with, and incorporated by reference into, Provisional priority application 60/076102 filed 26 Feb 1998. In particular text on pp. 24-25 of the unpublished manuscript is now also contained in the present application and PCT parent under the title: <u>Importance of disequilibrium and marker heterozygosity (i.e. marker allele frequency) in detecting linkage.</u> The unpublished manuscript, including its important Equation 2, Table 2 and pages 24-25 is referred to in the present application (see p. 39 line 39, 40 lines 7, 9-10, 17-18) and in the PCT parent (see p. 38 lines 25, 33, 36; and p. 39 lines 7-8). The unpublished manuscript is also referred to in Provisional priority application 60/076102 filed 26 Feb 1998 (see 60/076102 p. 12 lines 12-14, p. 13 lines 10-16, lines 44-46, p. 37 lines 46-55, p. 81 line 56 to p. 82 line 14, and p. 125 lines 2-4). The unpublished manuscript (included with, and incorporated by reference into, 60/076102) is entitled <u>Detection of linkage: Comparison of the affected sib pair (ASP) test and transmission/disequilibrium test (TDT)</u>.

As requested in the last sentence of p. 9 of the Office Action of 9/22/08, the applicants' arguments will refer only to the specification as filed. **The applicants respectfully**

thank the Examiner for also supplying the applicants with a copy of priority parent application PCT/US99/04376 in the last Office Action.)

Page 39 line 15 to p. 42 line 3 of the originally filed present application contains Table 2 (p. 41). In fact Table 2 contains the calculations that show the unexpected results that the Examiner states are not present in the application. The calculations in Table 2 are based on **Equation 2 for P_t** (top p. 40). The significance of P_t is described on page 40 lines 4-6: *"P_t may be regarded as the size of the 'signal' which is given by the TDT…. The more P_t is elevated above 0.5 (baseline), the greater is the evidence for linkage or 'power' provided by the association-based linkage test known as the TDT."*

Equation 2 is the exact same Equation 2 in the inventor's published paper in the Annals of Human Genetics vol 62, pp. 159-179, 1998 (see p. 39 lines 38-40). (Annals of Human Genetics vol 62, pp. 159-179, 1998 is referred to herein as AHG98. AHG98 is incorporated by reference into the current application, see p. 49 lines 21-23. AHG98 is also incorporated by reference into parent PCT/US99/04376, see PCT p. 48 lines 9-12.) An explanation of the significance of the calculations in Table 2 for the power of linkage studies using bi-allelic markers is contained in the application on, for example, p. 40 lines 7-23.

More specifically in Table 2 the disease allele's frequency is fixed at p=0.1 while the frequency (m) of the positively associated marker allele varies (m = .5, .3, .2, .1, .05) see p. 40 lines 10-12. **For a fixed level of disequilibrium (or association) the closer the bi-allelic marker allele frequency (m) is to 0.1, the frequency of the disease allele p (p = 0.1), the higher value of P_t (see p. 40 lines 12-17).**

This means that when the disease allele frequency p is 0.1 (or a similar low value), then the value of P_t is higher for bi-allelic markers with lower allele frequencies such as m = 0.2 or 0.1 than the value of P_t when m = 0.5; see p. 40 lines 17-23 comparing P_t

and statistical significance when m = 0.5 with P_t and statistical significance when m = 0.2.

The actual calculations referred to above on p. 40 lines 17-23 are under the heading **Importance of disequilibrium and marker heterozygosity (i.e. marker allele frequency) in detecting linkage** (p. 42 lines 5-6). These actual χ^2_{tdt} calculations (yielding 11.54 and 3.61) comparing power when m = 0.2 and m = 0.5 (for p = 0.1 and disequilibrium of $\delta = \frac{1}{2}\delta_{max}$) are on page 43 lines 14 to 24. (These actual χ^2_{tdt} calculations are in parent PCT/US99/04376, see PCT p. 42 lines 14 to 24.)

As noted in the calculations the χ^2_{tdt} result for m = 0.5 is not statistically significant ($p < 0.1$), **i.e., little or no power.** The χ^2_{tdt} result for m = 0.2 is statistically significant ($p < 0.005$), **i.e., significant power. As explained in the Background of the patent application the inventor's result is completely contrary to the understanding about marker allele frequency (m) and linkage study power that was present in the conventional art. In the conventional art m = 0.5 gives the highest power, in the inventor's example calculations m = 0.5 gives the lowest power.**

The conventional art's view of bi-allelic marker allele frequency and linkage study power is described, for example in the Background on p. 4 line 35 to p. 5 line 23 (corresponds to PCT p. 4 line 9 to p. 5 line 9) in reference to Kruglyak (1997). More specifically *"For bi-allelic markers his* [Kruglyak 1997] *results showed that the optimum allele frequencies for bi-allelic markers used in linkage studies is 0.5/0.5 in order to achieve the greatest information content."* And similarly p. 5 lines 10-11 (PCT p. 4 lines 33-34) states: *"The greatest information content is given by bi-allelic markers with allele frequencies close to the optimum of 0.5/0.5."* (As a person of ordinary skill in the art knows, "informativeness", "information content" and "power" are essentially

equivalent in this context.) **It is clear then that Kruglyak (1997) and the other conventional art "teaches away" from the invention.**

The inventor's original contention of the importance of using lower frequency bi-allelic markers in linkage studies is described on p. 8 lines 1-3 (PCT p. 7 lines 27-29); and this is contrasted with the Kruglyak (1997) reference p. 8 lines 3-5 (PCT p. 7 lines 29-31) that views lower frequency markers unfavorably. Again, it is clear that the conventional art "teaches away" from the invention. For example, the Examiner cited Cohen (U.S. 5,945,522 filed Dec. 22, 1997) in the obviousness rejection (see top p. 5) in the previous Office Action of 9/22/08. The applicants respectfully submit that Cohen (1997) is central to this obviousness rejection.

Yet Cohen (1997) clearly espouses the same conventional view as Kruglyak (1997) that strongly prefers higher minor (or least common) allele frequency bi-allelic markers with, for example, minor (least common) allele frequencies greater than or equal to 0.3. To confirm this see Cohen (1997) under the heading Linkage Disequilibrium, col. 10 line 62-63: *"Association studies have most usually relied on the use of bi-allelic markers."* And now see related text a short way further down Cohen (1997) at col. 11 lines 1-8: *"There are potentially more than 10^7 bi-allelic markers lying along the human genome.* ***However, a bi-allelic marker will show a sufficient degree of informativeness for genetic mapping only provided the frequency of its less frequent allele is not less than about 0.3, i.e., its heterozygosity rate is higher than about 0.42 (the heterozygosity rate for a bi-allelic marker is 2 P_a (1- P_a), where P_a is the frequency of allele a)."***

To confirm this further, see Cohen (1997) col. 16 lines 40-45: *"Given that the assessed distribution of informative bi-allelic markers in the human genome (bi-allelic polymorphisms with a heterozygosity rate higher than 42%) is one in 2.5 to 3 kb, six 500 bp genomic fragments have to be screened in order to identify 1 bi-allelic marker."* And now see related text a short way further down at Cohen (1997) col. 16 line 66 to

col. 17 line 6 (emboldening added): *"The detection limit for the frequency of bi-allelic polymorphisms detected by sequencing pools of 100 individuals is 0.3.+-.0.05 for the minor allele, as verified by sequencing pools of known allelic frequencies.* ***Thus, the bi-allelic markers selected by this method will be 'informative bi-allelic markers' since they have a frequency of 0.3 to 0.5 for the minor allele and 0.5 to 0.7 for the major allele, therefore an average heterozygosity rate higher than 42%.****"*

(For the Examiner's convenience the heterozygosity of a bi-allelic marker is, as stated in Cohen (1997) at col. 11 lines 6-8, 2 P_a (1- P_a), where P_a is the frequency of allele a. So for the heterozygosities for bi-allelic minor allele frequencies are as follows: P_a =0.1, Heterozygosity=0.18 or 18%; P_a =0.2, Heterozygosity=0.32 or 32%; P_a =0.3, Heterozygosity=0.42 or 42%; P_a =0.4, Heterozygosity=0.48 or 48% and P_a =0.5, Heterozygosity=0.5 or 50%. **Thus bi-allelic markers with the highest minor allele frequency of 0.5 have the highest heterozygosity. And bi-allelic markers with low minor allele frequencies, e.g., 0.1, have low heterozygosities.**)

The conventional art (such as Kruglyak (1997) and Cohen (1997)) have a strong preference for bi-allelic markers with the highest heterozygosity. This is because these highest heterozygosity markers were essentially thought to be the most "informative", have the highest "information content" and the most "power" for linkage studies.

The inventor, however, has turned these concepts on their head. Because, as stated above, in the conventional art m = 0.5 (highest heterozygosity) gives the highest power, in the inventor's example calculations m = 0.5 (highest heterozygosity) gives the lowest power. And in the inventor's example calculations m = 0.1 (lower heterozygosity) gives higher power.

The inventor discovered an unknown result effective variable for association-based linkage tests, see p. 44 lines 32-35; (this text also appears in the PCT parent and Provisional priority application 60/076102). This result effective variable is also

described as major finding (2) on p. 160 of the inventor's paper AHG98. Major finding (2) (emboldening added) of the author/inventor's investigation (AHG98) is: *"(2) TDT power is increased by disequilibrium between a bi-allelic marker and disease locus,* ***and is also <u>markedly increased when the disease allele and positively associated marker allele have similar population frequencies.</u>"*** (Result effective variables are discussed in MPEP 2141.02 III V and 2144.05 II B).

As further examples of the conventional art's preference for higher heterozygosity markers in linkage studies, see the Levinson reference, p. 3, footnote 2 in the present application (Levinson, et.al.: Genome Scan of Schizophrenia. Am J Psychiatry, June 1998; vol. 155: pp. 741-750). A copy of the Levinson reference was included as reference H in the Information Disclosure Statement for parent application 09/623068. Page 743, left mid column, of the Levinson reference describes a total map length of 3,410 centimorgans, with a mean spacing of 11 cM and **a mean marker heterozygosity of 76%.**

See also the Reed reference, p. 3, footnote 1 in the present application (Reed, et.al.: Chromosome-specific microsatellite sets for flourescence-based, semi-automated genome mapping. Nature Genetics, July 1994; vol. 7: pp. 390-395). A copy of the Reed reference was included as reference G in the Information Disclosure Statement for parent application 09/623068. Page 391, left mid column, of the Reed reference describes 254 microsatellite markers loci with an **average heterozygosity of 0.80 (97% of the markers had heterozygosity ≥ 0.70)**; see also the Abstract of the Reed reference.

See also the Kruglyak (1998) reference, p. 3, footnote 3 in the present application (Kruglyak, et. al.: Linkage Thresholds for Two-stage Genome Scans. Am J Hum Genet, 1998, vol. 62: pp. 994-996). A copy of this Kruglyak (1998) reference was included as reference I in the Information Disclosure Statement for parent application 09/623068. Page 995, right top mid column, of this Kruglyak (1998) reference

describes a simulation of linkage scans with markers assumed to have four equally frequent alleles (**each such marker having a heterozygosity 0.75**). See also p. 4 lines 29-30 of the present application which also describes this in this Kruglyak (1998) reference.

All these examples illustrate the preference in the conventional art for high heterozygosity markers, with heterozygosity near 0.75 or 75%, or a skewing of markers toward high heterozygosities (e.g., 0.75 or higher). These conventional marker maps and scans for linkage studies are not skewed toward, or preferential toward, the use of low heterozygosity markers such as bi-allelic markers with minor allele frequencies of 0.1 or 0.2 (heterozygosities of 0.18 or 0.32).

The applicants now respectfully further address the Examiner's statement that the *"applicant's assertion of unexpected results constitute mere argument"* (p. 9 of the Office Action of 9/22/08). As explained above results in Table 2 (p. 41; PCT p. 40) are based on Equation 2 (top p. 40; PCT p. 38 line 27) and the significance of the calculations is explained. Equation 2 in the application is the exact same Equation 2 in the inventor's published paper in the <u>Annals of Human Genetics</u> vol 62, pp. 159-179, 1998 (see p. 39 lines 38-40; PCT p. 38 lines 24-26); see top p. 162 of the published paper, referred to herein as AHG98.

A copy of AHG98 was supplied as Reference A in an Information Disclosure Statement (IDS) filed in November 2000 for parent application 09/623,068. <u>**A copy of AHG98 was also supplied in May of 2008 in the file for the present application.**</u> **AHG98 is a published paper in a very reputable, prestigious, refereed academic journal.** The author/inventor, R. E. McGinnis was associated with the Department of Genetics at the University of Pennsylvania School of Medicine (see top, page 159 AHG98). In addition, the author/inventor, R. E. McGinnis is a co-author of many articles in the field of Human Genetics, some of these papers are described, for

example, in the Background of the application, see p. 1 lines 13-14, p. 5 line 26 and footnote 7; PCT p. 48 lines 10-12, PCT p. 5 line 12 and footnote 7).

Equation 2 is based on a general algebraic framework and model of linkage as described in the Abstract and on page 161 of the published paper AHG98. The major findings of the AHG98 are given on the second page, page 160. Major finding (2) (emboldening added) of the author/inventor's investigation is: *"(2) TDT power is increased by disequilibrium between a bi-allelic marker and disease locus,* ***and is also <u>markedly increased when the disease allele and positively associated marker allele have similar population frequencies.</u>****"* This major finding leads to the inventor's advocating the use, for example, of bi-allelic markers with lower minor allele frequencies such as 0.3, 0.2 and 0.1 in linkage studies whose target disease (or trait-causing) polymorphism has a lower allele frequency, such as p = 0.1. **Again, major finding (2) of the author/inventor's investigation is in a published paper in a prestigious refereed academic journal.**

This published paper (AHG98) includes calculations (including power calculations) in Tables 1-3, pp. 165 & 167. The calculations in these tables support major finding (2) **<u>including the inventor's discovery of the importance of the similarity of disease (or trait-causing) allele and marker allele frequencies for the power of association-based linkage tests.</u>** As in the patent application(s) marker allele and disease allele frequencies are denoted "m" and "p" respectively and disequilibrium (or association) is given in terms of $\delta = \delta_{max}$ or $\delta = 1/2\delta_{max}$ in these Tables 1-3. As in the patent application(s) low frequency examples of "m" and "p" are given in Tables 1-3, pp. 165 & 167 of AHG98.These inventor calculations are cited in the Background of the present application (p. 6 lines 8-12) and in the Background of the PCT parent (p. 5 line 25 to p. 6 line 3).

Similarly there are numerous calculations in Tables 1 and 2 and Figures (graphs) in the inventor's unpublished manuscript that support major finding (2) **<u>including the</u>**

inventor's discovery of the importance of the similarity of disease (or trait-causing) allele and marker allele frequencies for the power of association-based linkage tests. The unpublished manuscript was included with (and incorporated by reference into) priority applications for the present application such as Provisional priority application 60/076102 filed 26 Feb 1998.

Page 164 of AHG98 describes the use of sample size n_{tdt} and binomial probability P_t to generate a binomial distribution. And as AHG98 states, standard tables giving the normal approximation to the binomial distribution (Pearson & Hartley, 1954; Weir 1996) provide precise power values for virtually any sample size (n_{tdt}), binomial probability (P_t) and significance level. **The description on page 164 and the general algebraic model in AHG98 allow TDT power calculations under a wide variety of conditions, e.g., for any values of "m" (allele frequency of bi-allelic marker), "p" allele frequency of presumed target disease (or trait-causing) polymorphism, penetrance ratio "r" and level of disequilibrium between the marker and disease (or trait-causing) polymorphism.**

The above description in refereed academic journal article AHG98 is incorporated by reference into both the present application and the PCT parent PCT/US99/04376. In addition, the general algebraic framework and model for linkage, the Equation 2 for P_t and the information on page 164 that allows for TDT power calculations under a wide variety of conditions is also present in the inventor's unpublished manuscript that was included with (and incorporated by reference into) priority applications for the present application such as Provisional priority application 60/076102 filed 26 Feb 1998. This inventor's unpublished manuscript was submitted for publication (to the American Journal of Human Genetics) in December of 1996, but was rejected (see p. 39 line 39; PCT p. 38 line 25).

Major finding (2) (which includes the importance of allele frequency) in AHG98 also leads to the statement from the unpublished manuscript that is in the present

application (see p. 40 lines 17-18 and p. 43 line 25 to p. 44 line 2; PCT p. 39 lines 7-8 and PCT p. 42 line 25 to p. 43 line 2): *"This example is typical, and highlights perhaps the most important finding of this paper; namely the importance of using bi-allelic markers with heterozygosity similar to that of a bi-allelic disease locus. Indeed, since a majority of susceptibility loci may be bi-allelic, the judicious use of bi-allelic markers of both high, medium, and low heterozygosity may be crucial in order to initially detect and replicate linkages to loci conferring modest disease risk."*

Yet the above statement in the unpublished manuscript about *"the most important finding of this paper"* was deemed by an academic Reviewer for the journal the American Journal of Human Genetics **to be of doubtful value.** See the comments of Reviewer B from some time on or before January 28, 1997. Page 2, second to the last paragraph of Reviewer B's review (Exhibit A, p.82) states: *"I also doubt that what the author calls 'perhaps the most important finding of this paper; namely the importance of using bi-allelic markers with heterozygosity similar to that of a bi-allelic locus' (page 25) is a* [sic] *advice really justified. This result is based on comparing the power of the TDT for different marker allele frequencies and δ_{max}, $\delta_{max}/2$ (Table 2). The author does not take into account the situation of a rare marker allele being in positive linkage disequilibrium with disease allele D may be quite infrequent. For example, consider a simplistic model where all disease carrying haplotypes are copies of the same ancestral mutated haplotype. It is unlikely that the original mutation occurred on a haplotype carrying the rare marker allele."*

The applicants respectfully submit that what is stated above by the Reviewer is an opinion of a lack of a reasonable expectation of success. This opinion of a lack of a reasonable expectation of success is particularly critical of, and discourages the use of, *"rare marker allele[s]"*, i.e. those bi-allelic markers with low minor allele frequencies. These bi-allelic markers with low minor allele frequencies are the very markers the inventor's work indicates are unexpectedly important. A reasonable expectation of success is required by American patent law to show obviousness, see

MPEP 2143.02 (Reasonable Expectation of Success is Required). On the strength of this unfavorable review and comments of another academic Reviewer, the unpublished manuscript was initially rejected for publication by the American Journal of Human Genetics, see the attached Exhibit A, pp. 78-82: a rejection letter from Peter Byers, editor of the American Journal of Human Genetics dated January 28, 1997 & associated Reviewer comments (total of four pages).

The identity of these Reviewers is unknown to the applicants. But the applicants respectfully submit that, as is customary, the academic Reviewers in this case at the American Journal of Human Genetics were high-quality conscientious Reviewers. **And the applicants respectfully submit that the reason the unpublished manuscript was initially rejected for publication is because the results in the unpublished manuscript, though correct, were so contrary to conventional thinking in the art of linkage studies at the time of review and were unobvious.**

Empirical Evidence regarding the physical extent of Linkage Disequilibrium

As is well known in the art the presence of linkage disquilibrium (LD), especially the presence of higher degrees of LD, is important for linkage studies based on association. As is also well known the degree of LD between polymorphisms generally decreases with increasing distance (or separation) between the polymorphisms. This quote, for example, is from U.S. Patent 5,945,522, Cohen (1997): *"The degree of disequilibrium dissipation depends on the recombination frequency, so the markers closest to the disease gene will tend to show higher levels of disequilibrium than those that are farther away (Jorde L B, 1995, Am. J. Hum. Genet. 56: 11-14)."* See column 13 lines 33-37 of Cohen (1997). This is the reason that smaller chromosomal location components of the covering distance are described as generally preferred in the Description, see the present application p. 27 lines 13-15 & lines 32-33.

Information on the physical extent of LD along the genome in various human populations based on empirical observations is given in the present application

on p. 27 lines 24-25 and p. 44 lines 22-25. More recent empirical observations obtained since the time of filing of the earliest priority application, Provisional priority application 60/076102 (filed 26 Feb 1998), are consistent with the information in the present application.

For example the paper Abstract of Reich, et. al (Nature. 2001 May 10;411(6834):199-204. Linkage disequilibrium in the human genome.) states: *"Here, we report a large-scale experiment using a uniform protocol to examine 19 randomly selected genomic regions. LD in a United States population of north-European descent typically extends 60 kb from common alleles… By contrast, LD in a Nigerian population extends markedly less far."*

The Abstract of a 2007 paper, Wang, et. al. (Genetics and molecular research 2007 Dec 11;6(4):1131-41. Increased gene coverage and Alu frequency in large linkage disequilibrium blocks of the human genome.) describes *"Very large LD blocks (>200 kb).."* The paper describes LD blocks separated by gaps of low LD. An LD block is described as *"a genomic region in which high LD is maintained"* (top p. 1132). Table 1 on p. 1134 of this paper gives information on the size of LD blocks in three human populations. In summary about 50% of blocks are 1-10 kb long, about 20% of blocks are 10-20 kb long, about 20% of blocks are 20-50 kb long, about 5% of blocks are 50-100 kb long, about 5% are 100-200 kb long and less than 1% are greater than 200 kb long.

In a 2007 paper, Kolkman, et. al, (Genetics. 2007 Sep;177(1):457-68. Single nucleotide polymorphisms and linkage disequilibrium in sunflower.) describes the extent of LD in plant species. For example, the paper describes LD persisting over genome tracts 400-1500 bp in maize and tracts > 50 kbp in soybeans (top left column p. 458). And LD is described as decaying within ~ 200 bp of wild sunflower alleles and ~ 1100 bp of exotic sunflower alleles (bottom right column p. 458). And LD expressed

in terms of r^2 only decayed from about 0.5 to 0.32 by 5500 bp in inbred sunflower populations (see p. 466 mid top left column and Figure 6 p. 465).

A 2007 paper (Du, et. al., Int J Biol Sci. 2007 Feb 10;3(3):166-78. Characterizing linkage disequilibrium in pig populations.) describes the extent of LD in domestic pig populations. For LD to reach a value of r^2 of 0.3 or above requires polymorphism (e.g., SNP) separation of less than about 0.3 cM (300,000 bp), higher values of r^2 (e.g., > 0.35) requires polymorphism (e.g., SNP) separation of less than about 0.1 cM (100,000 bp); see Figure 8 p. 174, Table 2 p. 175 and Discussion left upper column p. 177.

The applicants will supply the Examiner with a copy of the Abstract and the three papers cited above. It should be noted that the extent of LD described in the papers above (generally less than or equal to 200-300 kb) is LD that is “within the scope” of the presently pending claims. This is because the maximal value of “x,” the chromosomal location component of the covering distance, is greater than 200-300 kb in the pending claims. Specifically “x” is less than or equal to 1 million base pairs (e.g., claim 91), “x” is less than or equal to 250,000 base pairs (e.g., claim 109), and “x” is less than or equal to about 250,000 base pairs (e.g., claim 182).

Case law cited by the Examiner in the statement of lack of objective evidence of unexpected results

The Examiner’s obviousness rejection cites MPEP 716.01(c), In re Lindner, Ex parte George, and an *“absence of experimental data, tests or calculations,”* see bottom p. 9 of the Office Action of 9/22/08. Yet in In re Lindner the claimed inventions are in the chemical arts, specifically *“dispersant compositions.”* The Court, the Examiner and the Patent Office Board of Appeals in In re Lindner found *“the Rule 132 affidavit unpersuasive for a number of reasons, but basically it was their view that since only a single composition of those included in the claims was tested, the affidavit falls far*

short of establishing that the compositions encompassed by claims of the scope of the those on appeal possess unexpected properties."

The applicants respectfully submit that the situation in In re Lindner is not applicable to the present instant situation. For example, the claimed invention(s) in Lindner are in the chemical arts, which is a different kind of predictability than the general algebraic model of linkage, a mathematical model, used by the inventor in his genetics research. In addition, the inventor's discovery of the unexpected results of the importance of the similarity of marker and disease (or trait-causing) polymorphism allele frequencies for linkage study power is illustrated by the numerous calculations, initially negative and ultimately positive academic review, and the other evidence cited above.

The Examiner also cited Ex parte George, 21 USPQ2d 1058 in the obviousness rejection and an *"absence of experimental data, tests or calculations,"* see bottom p. 9 of the Office Action of 9/22/08. The claimed invention(s) in George were methods of coating metallic substrates with liquid crystal polymers. Again this is a different kind of art and predictability than the general algebraic model of linkage, a mathematical model, used by the inventor in his genetics research. In George, the Board of Patent Appeals and Interferences (BPAI) cited a lack of evidence supporting the inventor/declarant's contention of unexpected results. Specifically the Board stated: *"..it is thus unclear how results could be characterized as 'unexpected' since declarant does not refer to any recognized scientific principles to support opinion that reported results are unexpected, since it is impossible to determine whether declarant has presented fair comparison with closest prior art, and since there is no evidence that the two comparative polymers tested were actually used in 'real world' for coating metals,"* see Ex parte George, 21 USPQ2d at 1059, mid left column.

Again, this is not the case here and now in the present instant situation. The inventor's discovery of the unexpected results of the importance of the similarity of marker and disease (or trait-causing) polymorphism allele frequencies for linkage study power is

illustrated by the numerous calculations, initially negative and ultimately positive academic review, and the other evidence cited above. The applicants respectfully caution the Examiner regarding the applicability of Ex parte George and In re Lindner to the present situation and cite MPEP 2144 Supporting a Rejection Under 35 U.S.C. 103 III. Legal precedent can provide the rationale supporting obviousness <u>only if</u> the facts in the case are sufficiently similar to those in the application (underlining added).

<u>Summary of the rebuttal of Examiner statement of lack of objective evidence of unexpected results.</u>

The applicants have cited the abundance of evidence for the <u>unexpected, unobvious</u> property of increased power of lower minor allele frequency (lower heterozygosity) covering markers to detect linkage with an association-based linkage test. Thus the groups or subgroups of lower minor allele frequency (lower heterozygosity) covering markers delineated by whereby clauses in the presently pending claims have the <u>unexpected, unobvious</u> property of increased power to detect linkage in association studies. More specifically the increased power is to detect linkage (in association studies) to lower frequency trait-causing polymorphism alleles, such as a disease allele with a low allele frequency “p,” p = 0.1.

These “unexpected results” are important because (among other things) they make the prima facie case of obviousness (based on Cohen (1997) & Kruglyak (1995)) moot. This is because the groups/subgroups of lower minor allele frequency (lower heterozygosity) covering markers delineated by the “whereby clauses” in the pending claims cannot be “at once envisaged” (in light of Cohen (1997) & Kruglyak (1995)) by a person of ordinary skill in the art. A minor allele frequency subrange (“0.3 to 0.5” col. 17 lines 2-6 and “not less than about 0.3” col. 11 lines 2-5) described in Cohen (1997) that “touches” or “barely overlaps” a subrange (“less than or equal to 0.3”) present in <u>some claims</u> does not by itself establish obviousness. **Unless the groups/subgroups of covering markers (delineated by “whereby clauses”) can be “at once envisaged,” as required by the guidance of MPEP sections such as**

2131.02, 2131.03, 2144.05, and 2144.08 (e.g., 2144.08 II A 4. (a)), then the pending claims are not 102/103 obvious.

Further rebuttal of the obviousness rejection in the recent Office Action

The applicants now respectfully submit further remarks with respect to the obviousness rejection in the previous Office Action of 9/22/08. The applicants have reviewed the obviousness rejections of the previously pending claims under 35 USC 103 pp. 4-9. And the applicants have already respectfully submitted remarks above on a part of these obviousness rejection(s).

In the remarks above the applicants have indicated that later filed references cited by the Examiner in the recent obviousness rejections (specifically McGinnis et. al. (PCT/US/99/04376, WO/1999/43858) and Cohen (EP 0892068; Date of Publication: 20 Jan 1999) are not "prior art" against former claim 91 or the presently pending claims. This is because supporting subject matter for former claim 91 and the presently pending claims is present in the PCT parent, PCT/US99/0436, and in Provisional priority application 60/076102 (filed 26 Feb 1998). The applicants will continue to cite such supporting subject matter in the PCT parent and Provisional priority application in order to show that these later filed references (PCT/US/99/04376 and Cohen (EP 0892068; 1999)) are not prior art with respect to the presently pending claims.

Rebuttal of the case of prima facie obviousness based on the Cohen (1997) and Kruglyak (1995) references

Summary: The applicants will now submit evidence that Cohen (1997) and Kruglyak (1995) (as whole references) lead away from the claimed invention(s). Cohen (1997) does describe minor allele frequency subranges ("0.3 to 0.5" col. 17 lines 2-6 and "not less than about 0.3" col. 11 lines 2-5) that touch and barely overlap a subrange ("less than or equal to 0.3") present in some claims, see "whereby clauses" claims 91-93, 105, 171, 172, 201, 212-213. But this description alone does not establish obviousness.

Remarks in regard to reference Cohen et. al. (US 5,945,522 issued Aug. 31, 1999, Filed Dec. 22, 1997) used in the obviousness rejection. This Cohen (1997) reference is cited extensively by the Examiner in the obviousness rejection on pp. 5-9 of the Office Action of 9/22/08. The applicants wish to thank the Examiner of his close and conscientious reading of parts of the Cohen (1997) reference, but the applicants respectfully submit that the Examiner has misinterpreted his reading of parts of this reference.

More specifically the Examiner states (bottom p. 6): *"... Cohen (1997) shows lowest allele frequencies of bi-allelic polymorphisms below 1% (i.e. rare mutations) in the human genome and minor allele of 0.16 [Col. 52, Table 1]. One of ordinary skill in the art would have been motivated to include the rarest alleles (i.e. least allele frequencies less than 0.1) in order to create a more robust genotyping test by including with fewer false positive results."* **The applicants respectfully but strongly disagree, the reference does <u>not</u> in fact motivate one of ordinary skill in the art to genotype (or perform haplotype analysis with) markers of lower least common (minor) allele frequencies, for example with minor allele frequencies less than 0.3.**

The fact Cohen (1997) does not so motivate is clear from a closer reading of the reference. The bi-allelic markers used in linkage studies of Cohen (1997) are **<u>first selected</u>** based on having minor allele frequencies that are greater than or equal to 0.3 <u>in a heterogeneous French population</u>. As Cohen (1997) states in col. 17 line 2-6 (emboldening added): *"Thus, the bi-allelic markers selected by this method will be 'informative bi-allelic markers' since **they have a frequency of 0.3 to 0.5 for the minor allele** and 0.5 to 0.7 for the major allele, **therefore an average heterozygosity rate higher than 42%**."* And a little way down the reference states in col. 17 line 12-15 (emboldening added): *"The population used in order to generate bi-allelic markers in the region of interest consisted of ca. 100 unrelated individuals **corresponding to a French heterogeneous population**."*

The lower minor allele frequencies, such as 0.16 in Table 1 col. 52 to which the Examiner refers, <u>are in populations other than the French heterogeneous population (referred to above)</u>. These lower minor allele frequencies are in a <u>population with</u>

prostate cancer and in a control population without prostate cancer. These lower minor allele frequencies in Table 1 are determined in the prostate cancer affected and unaffected populations **after the bi-allelic markers have already been selected to have minor allele frequencies greater than or equal to 0.3 in a heterogeneous French population. In other words these lower minor allele frequencies were not selected, but simply occurred in a different population during haplotype analysis in bi-allelic markers already selected to have minor allele frequencies greater than or equal to 0.3.** See Cohen (1997) col. 20 lines 10-22: *"Haplotype Analysis The allelic frequencies of each of the alleles of bi-allelic markers 99-123, 4-26, 4-14, 4-77, 99-217, 4-67, 99-213, 99-221, and 99-135 (SEQ ID NOs: 21-38) were determined in the affected and unaffected populations. Table 1 lists the internal identification numbers of the markers used in the haplotype analysis (SEQ ID NOs: 21-38), the alleles of each marker, the most frequent allele in both unaffected individuals and individuals suffering from prostate cancer, the least frequent allele in both unaffected individuals and individuals suffering from prostate cancer, and the frequencies of these alleles in each population."*

The Examiner also states near bottom p. 5: *"Cohen (1997) teaches major and minor allele frequencies are determined, wherein the frequency of bi-allelic polymorphisms detected is 0.3+/0.05 for the minor allele [Col. 10, last ¶ and Col. 11, first ¶]."* In fact the figure *"0.3.+-.0.05"* the Examiner refers to is from Cohen (1997) col. 16 line 66 to col. 17 line 6 and states (emboldening added): *"The detection limit for the frequency of bi-allelic polymorphisms detected by sequencing pools of 100 individuals is 0.3.+-.0.05 for the minor allele, as verified by sequencing pools of known allelic frequencies.* ***Thus, the bi-allelic markers selected by this method will be 'informative bi-allelic markers' since they have a frequency of 0.3 to 0.5 for the minor allele and 0.5 to 0.7 for the major allele, therefore an average heterozygosity rate higher than 42%.****"*

The detection limit for the frequency of the minor allele of 0.3.+-.0.05 referred to above does not describe an intention to select for bi-allelic markers with minor allele

frequencies as low as 0.25 (i.e., 0.3-0.05 = 0.25). It simply means the detection test has limitations, which are based on several factors, including the number of individuals (100) in the pools. Thus, again, the Cohen (1997) reference as a whole does not motivate a person of ordinary skill to select bi-allelic markers with minor allele frequencies less than 0.3, as is clear from a plain reading of the text cited above. The applicants respectfully caution the Examiner that: *"A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. W.L. Gore & Associates, Inc. v. Garlock, Inc., 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984)"* see MPEP 2141.02 VI. **The applicants respectfully submit that Cohen (1997) as a whole does indeed lead away from the claimed invention(s).**

Similarly the Examiner statement (bottom p. 6): *"… Cohen (1997) shows lowest allele frequencies of bi-allelic polymorphisms below 1% (i.e. rare mutations) in the human genome"* **is not evidence of motivation** of the Examiner statement (also bottom p. 6) to "*include the rarest alleles (i.e. least allele frequencies less than 0.1) in order to create a more robust genotyping test by including with fewer false positive results."* Simply because Cohen (1997) mentions the existence of low minor allele frequencies, such as below 1%, *in various populations,* does not mean that the reference advocates the selection of bi-allelic markers with such low minor allele frequencies for linkage studies, genotyping and haplotype analysis. The reference does not advocate such a selection of bi-allelic markers. See for example the section of Cohen (1997) that mentions minor allele frequencies below 1% (rare mutations), col. 10 line 63 to col. 11 line 1. This section merely states: *"By definition, the lowest allele frequency of a bi-allelic polymorphism is 1%; sequence variants that show allele frequencies below 1% are called rare mutations."* But the very next passage in Cohen (1997) leads away from such markers and states (underlining added): *"There are potentially more than 10^7 bi-allelic markers lying along the human genome. However, a bi-allelic marker will show a sufficient degree of informativeness for genetic mapping only provided the frequency of its less frequent allele is not less than about 0.3, i.e., its heterozygosity*

rate is higher than about 0.42 (the heterozygosity rate for a bi-allelic marker is 2 P_a (1-P_a), where P_a is the frequency of allele a)." **Thus again, Cohen (1997) as a whole leads away from the claimed invention(s), see MPEP 2141.02 VI.**

Just above the passage (above) in Cohen (1997) in col. 16 that leads away from the invention is a passage the Examiner quotes on page 5 of the Office Action: *"Bi-allelic sites are systematically verified by comparing sequences of both strands of each pool (i.e. enabling selection of complementary markers) [See Col. 16 Example 2]."* The term "systematically" in this Cohen (1997) passage is, of course, much different than the use of the term "systematically" in the present application. And this passage also does not advocate selection of bi-allelic markers with lower minor allele frequencies, as evidenced by its proximity to the passage that leads away from the invention.

Oligonucleotides, the Cohen (1997) reference and the obviousness rejection.
The Examiner cites passages in Cohen (1997) to support the obviousness of oligonucleotides that are complementary to bi-allelic markers. For example, the Examiner refers (on page 5) to: *"… the invention provides oligonucleotides which are used to detect bi-allelic sites that are in linkage disequilibrium with the PG1 gene for use in determining the risk of prostate cancer [Col. 6, lines 5-25]."*

In fact, however, the passage quoted above, [Col. 6, lines 5-25], makes no mention of bi-allelic markers or sites. The closest this passage comes to describing oligonucleotides is as follows. This passage describes *"a method of obtaining an allele of the PG1 gene"* comprising *"contacting…an agent capable of specifically detecting a nucleic acid encoding the PG1 protein."* One aspect of the method *"comprises contacting the nucleic acid with at least one nucleic acid probe capable of specifically hybridizing to said nucleic acid encoding the PG1 protein." "Another embodiment of the present invention is a nucleic acid encoding the PG1 protein which is obtainable by the method described above."*

A reading of the rest of Cohen (1997) has failed to turn up one or more bi-allelic markers that encode the PG1 protein, as described in the passage above. In fact, parts of Cohen (1997) appear to indicate that finding a bi-allelic marker that is functional and encodes the PG1 protein is unlikely. See col. 11 lines 50-59: *"Association between a bi-allelic marker A and a trait T may primarily occur as a result of three possible relationships between the bi-allelic marker and the trait. First, allele a of bi-allelic marker A may be directly responsible for trait T (e.g., Apo E e4 allele and Alzheimer's disease). However, since the majority of the bi-allelic markers used in genetic mapping studies are selected randomly, they mainly map outside of genes.* ***Thus, the likelihood of allele a being a functional mutation directly related to trait T is therefore very low.****"* (Functional sequences are defined in col. 12 lines 45-49 to include exons. Exons are known to be the portions of the genome that encode proteins, see for example "exon" at http://www.genome.gov/glossary.cfm *"The region of a gene that contains the code for producing the gene's protein. Each exon codes for a specific portion of the complete protein."*)

"The Supreme Court in KSR noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit," see MPEP 2141 III Rationales to Support Rejections under 35 U.S.C. 103. The applicants respectfully request that the Examiner be more explicit in citing the parts of Cohen (1997) that describe or make obvious oligonucleotides that are complementary to bi-allelic markers as in the claimed invention(s).

Similarly the Examiner states (bottom p. 5) that Cohen (1997) describes nucleic acid arrays in Example 12 of Cohen (1997). But Example 12 describes arrays for *"Quantitative analysis of PG1 gene expression"* col. 37 lines 1-2, not genotyping arrays for genotyping at bi-allelic markers as in the pending claims. More specifically Example 12 gives specifics of the nucleic acids in these arrays (col 37 lines 8-10) as: *"The arrays may include the PG1 genomic DNA of SEQ ID NO:1, the PG1 cDNA of SEQ ID*

NO:3 or the sequences complementary thereto or fragments thereof." These fragments are then described (col 37 lines 10-18) lengths as at least 15 nucleotides in length, more preferably at least 100 nucleotides in length.

SEQ ID NO:1 is 56516 bases long, a very long sequence (see Sequence listing col 51) and SEQ ID NO:3 is 5227 bases long, also quite long (see Sequence listing col 102). It would appear then, that there are <u>many possibilities</u> within these sequences for selecting fragments of the length described in the above paragraph. But there is no specific description in Example 12 of oligonucleotides that are complementary to bi-allelic markers as in the claimed invention(s).

Again, *"The Supreme Court in KSR noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit,"* see MPEP 2141 III Rationales to Support Rejections under 35 U.S.C. 103. The applicants respectfully request that the Examiner be more explicit in citing the parts of Cohen (1997) that describe or make obvious arrays of oligonucleotides that are complementary to bi-allelic markers as in the claimed invention(s).

In summary the applicants respectfully submit that the Cohen (1997) reference as a whole leads away from, and does not render obvious, the claimed invention(s). There is no motivation in this reference for using oligonucleotides that are complementary to bi-allelic markers with low minor allele frequencies (and low heterozygosities). Oligonucleotides of the claimed invention(s) are for use in obtaining genotype data or sample allele frequency data at lower minor allele frequency (lower heterozygosity) bi-allelic markers such as those delineated by the various "whereby clauses" in the pending claims described above. **A person of ordinary skill in the art to cannot <u>immediately envisage</u> such oligonucleotides in light of Cohen (1997).** In addition, the applicants respectfully submit that the Examiner has not established a prima facie case of obviousness.

Remarks in regard to reference Kruglyak et. al. (Am. J. Hum. Genet., 1995, Vol. 57, p. 437-454) used in the obviousness rejection. This Kruglyak (1995) reference is cited by the Examiner in the obviousness rejection, top p. 5 and p. 7 to top 8 of the Office Action of 9/22/08. **The applicants respectfully submit that the Kruglyak (1995) reference, like Kruglyak (1997) and Kruglyak (1998) cited above, leads away from the claimed invention(s).** This Kruglyak (1995) reference, like the other conventional art references cited above, has no awareness of the result effective variable for association-based linkage tests discovered by the inventor, see for example, p. 44 lines 32-35 of the present application and major finding (2) on p. 160 of AHG98. Major finding (2) (emboldening added) of the author/inventor's investigation (AHG98) is: *"(2) TDT power is increased by disequilibrium between a bi-allelic marker and disease locus,* ***and is also <u>markedly increased when the disease allele and positively associated marker allele have similar population frequencies.</u>****"* (Result effective variables are discussed in MPEP 2141.02 III V and 2144.05 II B).

So the Kruglyak (1995) reference, like the other references cited above, focuses on high heterozygosity markers. This focus of the Kruglyak (1995) reference on high heterozygosity markers is evident from the captions of the Figures in Kruglyak (1995). Specifically in Figures 1, 2, and 3 marker heterozygosity is 0.8. In Figure 4, heterozygosity is 0.9, 0.8, and 0.5. In Figures 5 and 6, markers have 2, 3, 5, 10, 20 and 100 equally frequent alleles with heterozygosities of 0.5, 0.67, 0.8, 0.9, 0.95 and 0.99, respectively. Figure 7 refers to an actual study (Davies, et. al.Nature vol. 371 pp. 130-136) no heterozygosity is given. However, Figure 1 on p. 133 of Davies, et. al. indicates the markers used in the study were microsatellites from the marker map described in the Reed reference discussed above on p. 31 (Reed, et.al. Nature Genetics, July 1994; vol. 7: pp. 390-395), wherein the 254 microsatellite markers loci had an average heterozygosity of 0.80 (97% of the markers had heterozygosity ≥ 0.70). In Figure 8 marker heterozygosity is 0.8. The Kruglyak (1995) reference refers specifically to bi-allelic markers, but only to bi-allelic markers with the highest possible heterozygosity of 0.5 (minor allele frequency of 0.5, 2 equally frequent alleles), see

captions of Figures cited above and under Information Content and Study Design Considerations: The Quality of a Map mid right column p. 445 (*"perfect bi-allelic markers (50% heterozygosity)"*).

These high heterozygosities, i.e. greater than or equal to 0.5, are much greater than the heterozygosities of the bi-allelic covering markers delineated by the "whereby clauses" in the presently pending claims. The covering markers delineated by these "whereby clauses" have minor allele frequencies less than or equal to 0.3, 0.2 and 0.1. These minor allele frequencies correspond to heterozygosities of less than or equal to 0.42, 0.32 and 0.18 respectively.

It should be noted that the Kruglyak (1995) uses an **Information-Content Mapping** approach for marker selection (see Abstract p. 441 and right column p. 443 to p. 447). This **"Information-Content"** approach is essentially the same as (or very similar to) the "Information-Content" approach used in a later published reference by the same author Kruglyak (The use of a genetic map of biallelic markers in linkage studies. Nature Genetics, September 1997, vol.17, pp. 21-24) cited in the Background (footnote 4, p. 5) of the present patent application. That Information Content approach leads to bi-allelic markers with lower least common allele frequencies (e.g., less than 0.3) being viewed unfavorably. Specifically, to repeat what was stated above (with emphasis added), p. 8 lines 1-5 of the present application states, *"In addition, the inventor's calculations and observations indicate that bi-allelic markers having least common allele frequencies less than 0.3, 0.2 or even less than 0.1 have an important place in linkage studies using association based linkage tests.* ***This is markedly different than Kruglyak's information content evaluation of bi-allelic markers for use in linkage studies, in which bi-allelic markers with least common allele frequencies less than 0.3 or 0.2 are viewed unfavorably***."

The Kruglyak (1995) reference cited by the Examiner in the Office Action is cited in footnote 5 p. 5 of the present application. Both of these Kruglyak references (1995

&1997) and the Information Content approach is discussed on p. 4 line 35 to p. 5 line 23 of the present application and the applicants respectfully direct the Examiner to p. 4 line 35 to p. 5 line 23 and also again to p. 8 lines 1-5. These references include the same author, Kruglyak; and the concepts in the references for the art of linkage studies appear to be cumulative. As explained on p. 4 line 35 to p. 5 line 23, these references lead to bi-allelic markers for use in linkage studies with lower minor allele frequencies (e.g., less than 0.3) being viewed unfavorably. Given such a "teaching away" from markers with lower minor allele frequencies, the applicants respectfully submit that the Kruglyak (1995) reference cited by the Examiner in the Office Action, either alone or in combination with Cohen (1997), does not render the claimed invention(s) obvious.

The Examiner also refers to Kruglyak (1995) Figures 1, 2 and 3 and two-dimensional graphs that are similar to x-y graphs (p. 7 of the Office Action of 9/22/08). As noted above, each of these Figures 1, 2 and 3 refer to ***high*** marker heterozygosity of 0.8 and markers (e.g., microsatellites) with five equally frequent alleles. These markers are <u>not</u> bi-allelic markers; bi-allelic markers have only 2 alleles.

None of the y-axes in the graphs in these Figures is "allele frequency." It would not be obvious to make the y-axes in graphs in these Figures "allele frequency" or to use lower minor allele frequency markers such as those delineated by whereby clauses in the pending claims without knowledge of the result effective variable for association-based linkage tests the inventor discovered, see for example p. 44 lines 32-35, and major finding (2) on p. 160 of AHG98. Major finding (2) (emboldening added) of the author/inventor's investigation (AHG98) is: *"(2) TDT power is increased by disequilibrium between a bi-allelic marker and disease locus,* ***and is also <u>markedly increased when the disease allele and positively associated marker allele have similar population frequencies.</u>****"* (Result effective variables are discussed in MPEP 2141.02 III V and 2144.05 II B).

In summary the applicants respectfully submit that no prima facie case of

obviousness has been established with respect to former claim 91 or the presently pending claims. Each of both Cohen (1997) and Kruglyak (1995) lead away from the claimed invention(s). Both references have no awareness of the result effective variable discovered by the inventor, as evidenced by their focus on high heterozygosity markers including higher heterozygosity (higher minor allele frequency) bi-allelic markers. These references would not it make obvious (to a person of ordinary skill in the art) to select lower minor allele frequency bi-allelic markers (such as those delineated in whereby clauses in the claims) for use in linkage studies. Nor would these references make it obvious to select oligonucleotides that are complementary to such lower minor allele frequency bi-allelic markers for use for example in genotyping.

Support for limitations in the presently pending claims to ensure compliance with the Written Description Requirement of 35 U.S.C. 112.

The applicants have already cited support for the limitation *"thousands of bi-allelic markers"* in the presently ending claims. This support was present in each of the present application, PCT parent application (PCT/US99/04376) and in Provisional priority application 60/076102.

The applicants will now cite support for limitations in the presently pending claims. In general the support will be cited for new, added limitations relative to the previous claim set of May 1, 2008, rather than for old, previously present limitations. The cited support will be in accordance with the Written Description Requirement; MPEP 2163 states in part: *"To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., Moba, B.V. v. Diamond Automation, Inc., 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563, 19 USPQ2d at 1116.*

The PCT parent, PCT/US99/04376 (filed 2/26/99), claims priority from Provisional priority application 60/076102 (filed 2/26/98). And each of the later filed parent U.S. National applications for the present application, 09/623068 filed 8/26/2000 and 09/947768 filed 9/5/2001, incorporate by reference the earlier filed PCT parent, PCT/US99/04376. The present application 10/037,718 also claims priority from Provisional priority application 60/076102.

Cited support for the limitations in the presently pending claims will cite specific parts of the present application 10/037,718, the PCT parent PCT/US99/04376, and Provisional priority application 60/076102. In most or all cases the page and line numbers of the relevant sections of these applications will be cited. **For the sake of brevity the following abbreviations will be used:** "'718 app." for the present application 10/037,718, "PCT" for the PCT parent PCT/US99/04376, and "Prov. '102" for Provisional priority application 60/076102.

Support for limitations in claim 91

<u>Remarks in support of claim 91 are longer and more detailed, Remarks in support of the other (dependent) claims are generally shorter and less detailed.</u>

For support for the added limitation *"wherein the set of oligonucleotides is selected for the set's utility to determine genotype data or sample allele frequency data for each of the two or more covering markers"* see '718 app. p. 37 lines 8-22, PCT p. 36 lines 3-17, and Prov. '102 p. 64 lines 42-50. (It should be noted that genotype data for *"samples of individuals"* that are *"groups of individuals who have supplied phenotype data regarding the genetic characteristic and provided chromosomal DNA samples which have been pooled"* is sample allele frequency data; see for example Prov. '102 p. 36 lines 23-34.)

For support for the added limitation *"wherein the group of covering markers is chosen so that a CL-F region is N-covered to within [x, y] by the covering markers,*

wherein [x, y] is a two-dimensional distance....N is an integer greater than or equal to 1." This limitation was present in dependent claims in the previous claim set filed May 1, 2008, so it really is not new. For support see for example, '718 app. p.14 lines 26-28, lines 33-37, PCT p. 13 lines 26-28, lines 33-37, and Prov. '102 p. 30 lines 45-47, lines 52-53, & p. 31 lines 2-4.

For support for the limitation *"wherein x is less than or equal to 1 million base pairs and y is less than or equal to 0.2"* see for example '718 app. p.27 lines 20-23, lines 27-28, 32-33, p. 29 lines 16-17; PCT p. 26 lines 11-14, lines 18-20, p. 28 lines 7-8 and Prov. '102 p. 35 lines 14, 20-22, 44-46, p. 40 lines 18-20.

For support for the limitation ***"****wherein N is less than maximal, whereby the number and distribution of known markers in the neighborhood of the CL-F region make it possible for N to be a greater value"* see '718 app. p.27 lines 36-37; PCT p. 26 lines 2-28 and Prov. '102 p. 35 lines 47-49. Each of these passage describes a maximal or largest value of N. Thus choosing a value of N that is <u>not</u> the maximal value of N (less than maximal) is described; see MPEP 2173.05(i) <u>Negative Limitations:</u> *"If alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. In re Johnson, 558 F.2d 1008, 1019, 194 USPQ 187, 196 (CCPA 1977) ('[the] specification, having described the whole, necessarily described the part remaining.')."*

For support for the added limitation *"whereby each point in the region is within the distance [x, y] of each of N or more of the covering markers."* This whereby clause is not a true limitation, it follows from the other limitations in the claim and the definition of N-covering. See '718 app. p. 13 lines 34-36 & p. 14 lines 26-28, PCT p. 12 lines 34-36 & p. 13 lines 26-28 and Prov. '102 p. 30 lines 19-21, lines 45-47.

For support for the limitation *"wherein the CL-F region is a segment-subrange."* This limitation was present in the previous claim set of May 1, 2008, so the limitation is not

new. For support see '718 app. p. 15 lines 19-20, p. 28 lines 4-5; PCT p. 14 lines 19-20, p. 26 lines 34-35 and Prov. '102 p. 27 lines 15-17, p. 40 lines 44-48, p. 72 lines 37-38. The whereby clause "*whereby the segment-subrange is a rectangular region on the CL-F map"* is not a true limitation. This whereby clause follows from the Description, see '718 app. p. 15 lines 19-20, PCT p. 14 lines 19-20 and Prov. '102 p. 27 lines 15-17. The limitation *"whereby the segment-subrange is bounded by a chromosomal segment and a least common allele frequency subrange"* is not a true limitation, but follows from the definition of segment-subrange; see '718 app. p. 15 lines 17-33, PCT p. 14 lines 17-33, and Prov. '102 p. 27 lines 3-38, and p. 31 lines 5-7.

For support for the added limitation *"wherein the length of the segment of the segment-subrange is greater than or equal to the length of human chromosome 21."* The application, PCT parent and Provisional priority application describe the segment of a segment-subrange as any length up to the length of an entire chromosome. And individual human chromosomes 1-22, X and Y are described. An example of the chromosomal location coordinates of CL-F points ranging over an entire chromosome, e.g. chromosome number 6, is given. Frequency subranges and chromosomal segments are described. See for example, '718 app. p. 14 lines 2-6, lines 10-13, p. 38 lines 29-30, p. 44 lines 26-27; PCT p. 13 lines 2-6, lines 10-13, p. 37 lines 15-16, p. 43 lines 26-27 and Prov. '102 p. 30 lines 27-30, lines 38-39, p. 40 lines 44-48, and p. 72 lines 37-38.

Each of chromosomes human chromosomes 1-22, and X and Y, including human chromosome 21, is thus an example of a described possible segment (and segment length) of a segment-subrange. The length of each of these example chromosomes is greater than or equal to the length of human chromosome 21, the shortest human chromosome. As stated above, the segment of a segment-subrange is described as any length up to the length of any chromosome. **And the example length of human chromosome 21 then acts as a described "range endpoint" for segment length as in the case In re Wertheim.** See for example, MPEP 2163.05 III. Range

Limitations *“In the decision in In re Wertheim, 541 F.2d 257, 191 USPQ 90 (CCPA 1976), the ranges described in the original specification included a range of ‘25%-60%’ and specific examples of ‘36%’ and ‘50%.’ A corresponding new claim limitation to "at least 35%" did not meet the description requirement because the phrase "at least" had no upper limit and caused the claim to read literally on embodiments outside the "25% to 60%" range, however a limitation to ‘between 35% and 60%’ did meet the description requirement.”*

For support for the added limitation *“whereby the length of the segment is greater than or equal to about 47 million base pairs”* is not a true limitation, but follows from the limitation immediately above. The length of human chromosome 21 is known to be at or about 47 million base pairs. Human chromosome 21 is also known to be the shortest human chromosome. The applicants will supply the Examiner with information from the National Library of Medicine and NIH that was reviewed in June 2008 that says: *“Chromosome 21 is the smallest human chromosome, spanning about 47 million base pairs.”* An estimate of a length of 51 million base pairs for human chromosome 21 is given and a statement that it is *“the smallest of human autosomes”* (p. 119, vol. 2) in the 1996 Edition of <u>Encyclopedia of molecular biology and molecular medicine,</u> Editor Robert A. Meyers. An estimate of 46 cM is given (p. 563, vol. 3) in the <u>Encyclopedia of molecular cell biology and molecular medicine</u> (2nd Edition, 2004) Editor Robert A. Meyers.

For support for the added limitation *“wherein the subrange of the segment-subrange includes the least common allele frequency 0.1,”* A polymorphism with least common allele frequency p = 0.1 has a very prominent role in the description as a sought (or target) disease (or trait-causing) polymorphism that is sought by two-dimensional association based linkage studies described in the application. For example as noted above, all of the calculations in Table 2 are based on p = 0.1. See Theory of Operation Sections of ‘718 app. p. 41, p.42 line 3, PCT p. 40, p. 41 line 3, and Prov. ‘102 p. 37 lines 24 & lines 46-55, p. 38 lines 7-8 and p. 82 lines 8-10. More

specifically in Table 2 the disease allele's frequency is fixed at p=0.1 while the frequency (m) of the positively associated marker allele varies (m = .5, .3, .2, .1, .05) see Theory of Operation Sections of '718 app. p. 40 lines 10-12, PCT p. 39 lines 1-2, and Prov. '102 p. 37 lines 24 & lines 46-55, p. 38 lines 7-8 and p. 82 lines 8-10.

This description also leads to the description of using lower heterozygosity (lower minor allele frequency, less than or equal to about 0.3, that are "close to 0.1"), markers, rather than just higher heterozygosity markers. See for example '718 app. p. 44 lines 1-2, PCT p. 43 lines 1-2, and Prov. '102 p. 14 lines 14-16.

Thus it is clear that the '718 app., PCT and Prov. '102 describe the least common allele frequency p = 0.1 as a place to look for a trait-causing (e.g., disease) polymorphism on a CL-F map. The present application also describes *"... a rectangular CL-F region, a segment-subrange, that is N covered is used in an association based linkage study to test for the presence of a trait causing bi-allelic gene located within the segment-subrange."* See '718 app. p. 28 lines 5-6, PCT p. 26 lines 34-36, and similar concepts are in Prov. '102 p. 16 lines 49-50, p. 17 lines 4-7, p. 20 lines 2-6. Therefore the present application, (and PCT parent and priority applications) describe a CL-F region that is a segment-subrange that contains the least common allele frequency p = 0.1. (It should be noted the terms "gene" and "trait-causing polymorphism" mean the same thing, see '718 app. p. 1 lines 34-36, PCT p. 1 lines 20-22, and Prov. '102 p. 25 lines 15-19.)
As an example, the Theory of Operation, set/subset example(s) describe covering rectangular CL-F region(s), segment-subrange(s), that include the least common allele frequency p = 0.1; see for example '718 app. pp. 44-47, PCT pp. 43-46, and Prov. '102 p. 75 line 26 to p. 76 line 50, especially lines 36-50.

For support for the added limitation *"whereby there are at least about 24 covering markers with least common allele frequencies less than or equal to 0.3 that are distributed within the segment with a density of at least about 1 marker every two*

million base pairs," this "whereby clause" is not a true limitation but necessarily follows from other limitations in the claim.

The least common allele frequencies of covering markers in the whereby clause being less than or equal to 0.3 necessarily follows because *"y is less than or equal to 0.2"* and the segment-subrange contains the minor allele frequency $p = 0.1$. That is, $p + y \leq 0.3$.

The density of covering markers in the whereby clause being at least about 1 marker every two million base pairs necessarily follows because *"x is less than or equal to 1 million base pairs."* The minimum density of the markers distributed within the segment in the whereby clause is equal or about equal to 1 marker every 2 times x; 2 times x is 2 million base pairs. This yields a minimum density of at least, or at least about, 1 marker every 2 million base pairs.

The number at least about 24 covering markers in the whereby clause necessarily follows from dividing the length of human chromosome 21 (about 47 million base pairs) by the density (about 1 marker every 2 million base pairs). This yields the number about 23.5. Since there is no such thing as 1/2 of a marker, the number is rounded up to 24.

Support for limitations in claim 92 There are no added limitations to claim 92 compared to previous claim 93 in the previous claim set of May 1, 2008.
Support for limitations in claim 93 There are no new limitations to claim 93 compared to previous claim 93 in the previous claim set of May 1, 2008.
Support for limitations in claim 104 There are no new true limitations to claim 104 compared to previous claim 104 in the previous claim set of May 1, 2008. The subrange of the segment-subrange is given as the subrange 0 to 0.5 in amended claim 104. This is supported, for example, by '718 app. p. 14 lines 10-13, PCT p. 13 lines 10-13, and Prov. '102 p. 30 lines 36-40.

The added whereby clause is not a true limitation, but follows from other limitations in the claim. The added whereby clause in claim 104 is almost identical to an added whereby clause discussed above in claim 92 (*"less than or equal to 0.2"* is substituted for *"less than or equal to 0.3"*). This substitution follows from the fact that y is less than or equal to 0.2 and the minor allele frequency p = 0 is included in subrange (0 to 0.5) of the segment-subrange that is the CL-F region. That is, $p + y \leq 0.2$.

Support for limitations in claim 105 There is an added limitation *"wherein the subrange of the segment-subrange is the subrange 0.1 to 0.2"*. This subrange is supported, for example, by '718 app. p. 14 lines 12-13, PCT p. 13 lines 12-13, and Prov. '102 p. 72 lines 18-54, especially line 48.

Support for limitations in claim 108 Only one true limitation is added to the claim 108 compared to previous claim 108 in the claim set of May 1, 2008. More specifically the subrange (of the segment-subrange) has been made slightly larger by changing the subrange *"0 to less than 0.1"* to simply *"0 to 0.1."* This is supported by the fact that the minor allele frequency p = 0.1 plays such an important role in the application(s) as discussed above. In effect the subrange $p < 0.1$ has been added to p = 0.1 to get the subrange $p \leq 0.1$. This is naturally supported and supported by Theory of Operation sections '718 app. p. 43 line 2, p. 45 lines 15-16, PCT p. 42 line 2, p. 44 lines 15-16, and Prov. '102 p. 38 lines 7-8, & p. 82 lines 19-20. These passages describe the possibility of the allele frequency "p" of the sought disease allele D being equal to 0.1 or less than 0.1 (*"below 0.1/above 0.9"*).

The added whereby clause in claim 108 is not a true limitation, but follows from other limitations in the claim. This whereby clause is identical to the whereby clause in claim 104. And this clause follows for essentially the same reasons as in claim 104: the fact that y is less than or equal to 0.2 and the minor allele frequency p = 0 is included in subrange (0 to 0.1) of the segment-subrange that is the CL-F region. That is, $p + y \leq 0.2$.

Support for limitations in claim 109 there are no new true limitations added to claim 109. The claim contains the limitation *"wherein x is less than or equal to 250,000 base pairs."* Support for this limitation was discussed on pp. 29 & 30 of the Preliminary Amendment of 10/2/07 and is discussed again now. See '718 app. p. 27 lines 13-15, lines 29-33, and examples p. 29 lines 16-17, p. 37 line 1, PCT p. 26 lines 12-14, lines 20-24, and examples p. 28 lines 7-8, p. 35 lines 34-35 and Prov. '102 p. 35 lines 23, 45-47 and examples p. 40 lines 19-20, and p. 43 line 42. (To further clarify, the examples use exact values for covering distances. And since smaller values of chromosomal location covering distance "x" are preferred, then *"x is less than or equal to 250,000 base pairs"* is preferred over *"x is less than or equal to 1 million base pairs"* (see claim 91)).

The added whereby clause is not a true limitation, but follows from the limitations in the claim. This whereby clause is identical to the whereby clause in claim 108, except that "96 covering markers" is substituted for "24 covering markers" and "1 marker every five hundred thousand base pairs" is substituted for "1 marker every two million base pairs." These two substitutions follow from the fact that the upper limit of "x" in claim 109 (250, 000 bp) is 1/4 of the value of the upper limit of "x" in claim 108 (1 million bp, see claim 91). So the approximate minimum number of markers in the whereby clause of claim 108 (24) is multiplied by 4 to yield 96. And the approximate minimum density of markers in the whereby clause of claim 108 (1 marker every 2 million bp) is also multiplied by 4 to yield "1 marker every five hundred thousand base pairs."

Support for the limitations in new claims 167-235 Most of the true limitations in these new claims are not new, but were limitations in the claims of the previously examined claim set of May 1, 2008. Support for these claims (under the Written Description Requirement of 35 U.S.C. 112) was previously cited in the Preliminary Amendment of October 2, 2007. For the Examiner's convenience, however, the

applicants may cite support that was already cited (in the Preliminary Amendment of 10/2/07).

Support for the limitation in new claim 167 Claim 167 contains the true limitation *"wherein y is 0.1."* This is supported for example by '718 app. p.27 lines 21-23, 29-31 and examples such as p. 29 lines 16-17, p. 37 line 21, PCT p. 26 lines 11-14, and examples such as p. 28 lines 7-8, p. 36 line 16 and Prov. '102 p. 35 lines 14-16, 44-46, and examples such as p. 40 lines 18-20. (To further clarify, the examples use exact values for covering distances. And since smaller values of frequency distance are preferred, then *"y is 0.1"* is preferred over higher values such as *"y is less than or equal to 0.2"* in claim 92.*)*

The whereby clause in new claim 167 is not a true limitation. The whereby clause in new claim 167 is almost identical to the whereby clause in claim 108, except that *"less than or equal to 0.2"* has been replaced by *"less than or equal to 0.1."* This follows from the fact that y is 0.1 and the minor allele frequency $p = 0$ is included in subrange (0 to 0.1) of the segment-subrange that is the CL-F region. That is, $p + y \leq 0.1$.

Support for the limitation in new claim 168 Claim 168 contains the true limitation *"wherein N is greater than 2."* Support for this limitation was previously cited in the Preliminary Amendment of 10/2/07 (p. 30). The applicants will now, however, cite the support again. Higher values of "N" are preferred, see '718 app. p.27 lines 33-34 and the example of $N \geq 2$ p. 29 lines 17-18, PCT p. 26 lines 24-25, and the example of $N \geq 2$ p. 28 lines 8-9 and Prov. '102 p. 35 lines 46-47, and the examples of $N \geq 2$, p. 11 line 40, p. 41 line 27, and p. 84 line 23. Since higher values of N are preferred and examples of N greater than or equal to 2 are given, then *"N greater than 2"* is supported.

The whereby clause in claim 168 is not a true limitation, but follows from limitations in the claim. The whereby clause in claim 168 is almost identical to the whereby clause in claim 109, except that *"96 covering markers"* has been replaced by *"288 covering*

markers." And *"1 marker every five hundred thousand base pairs"* has been replaced by *"1 marker every 167 thousand base pairs."* These two replacements follow from the fact that "N" in claim 168 is greater than 2 (i.e., N ≥ 3) and N ≥ 1 in claim 109 (see claim 91). So the approximate minimum number of markers in the whereby clause of claim 109 (96) is multiplied by 3 to yield *"288."* And the approximate minimum density of markers in the whereby clause of claim 109 (1 marker every 500,000 bp) is also multiplied by 3 and rounded up to yield *"1 marker every 167,000 base pairs."*

Support for the limitation in new claim 169 Claim 168 contains the true limitation *"x is less than or equal to 250,000 base pairs."* This limitation was discussed above under claim 109. The whereby clause in claim 169 is not a true limitation. This whereby clause is almost identical to the whereby clause in claim 109, except that *"less than or equal to 0.1"* replaces *"less than or equal to 0.2."* This replacement follows from the fact that *"y is 0.1"* in claim 169 (see claim 167) and *"y is less than or equal to 0.2"* in claim 109 (see claim 92).

Support for the limitation in new claim 170 This claim contains the true limitation *"N is greater than 2"* which is discussed above under claim 168. The whereby clause in claim 170 is not a true limitation. This whereby clause is similar to the whereby clause in claim 169, except that *"96 covering markers"* has been replaced by *"288 covering markers."* And *"1 marker every five hundred thousand base pairs"* has been replaced by *"1 marker every 167 thousand base pairs."* These two replacements follow from the fact that "N" in claim 170 is greater than 2 (i.e., N ≥ 3) and N ≥ 1 in claim 169 (see claim 91). So the approximate minimum number of markers in the whereby clause of claim 169 (96) is multiplied by 3 to yield *"288."* And the approximate minimum density of markers in the whereby clause of claim 169 (1 marker every 500,000 bp) is also multiplied by 3 and rounded up to yield *"1 marker every 167,000 base pairs."*

Support for the limitation(s) in new claims 171-178. Claims 171-178 contain the limitation *"wherein the chosen group of covering markers includes thousands of bi-*

allelic markers." Support of this limitation was already discussed above on pp. and in the Preliminary Amendment of Oct 2, 2007 (see for example, p. 19).

The applicants will now discuss some of this support again for the Examiner's convenience. The clause *"thousands of bi-allelic markers"* appears in connection with a way to implement the new Two-Dimensional Linkage Study Techniques of the present application. See '718 app. p. 25 lines 9-10, p. 35 lines 10-11 and p. 48 lines 1 to 16, especially line 5, PCT p. 24 lines 1-2, p. 34 lines 5-6 and p. 47 lines 1 to 16, especially line 5 and Prov. '102 p. 46 lines 7-15, and p. 53 lines 39-52, especially line 43. (Also, as noted above, the clause is also supported by parent 09/947,768, since 09/947,768 incorporates the PCT parent, PCT/US99/0436, by reference, see p. 68 line 34.)
The applicants respectfully submit that this support is further reinforced **for a person of ordinary skill in the art** by the context in the art at the time of filing of the present '718 app., PCT parent and priority Prov. '102. At the times of filing these applications, it was known in the art that thousands, even millions, of bi-allelic markers (SNPs) would likely become identified and become available for future use in linkage studies; see for example p. 21 mid right column of Kruglyak (The use of a genetic map of biallelic markers in linkage studies. Nature Genetics, September 1997, vol.17, pp. 21-24) that states *"classic estimates of more than 1 per 1,000 base pairs, or more than 3 million* [*S*NPs] *in the genome."* It should be noted that 3 million SNPs in the entire genome translates to about 130, 000 SNPs (3 million/23) per human chromosome.

This Kruglyak (1997) reference is cited in the Background of the present application, see footnote 4, p. 5. A copy of this Kruglyak (1997) reference was supplied as Reference F in an Information Disclosure Statement (IDS) filed in November 2000 for parent application 09/623,068. **The applicants will also expeditiously supply the Examiner with another copy of this Kruglyak (1997) reference (or any other reference referred to herein) for his convenience if he should so desire.**

The applicants respectfully submit that this support is further reinforced **for a person of ordinary skill in the art** by a reading of the Description that favors higher power embodiments of the invention with smaller values of the covering distance (δ) and higher values of N, including the largest possible value of N (see '718 app. p. 27 lines 32-39, PCT p. 26 lines 23-30, and priority Prov. '102 p. 35 lines 44-49). Smaller values of the covering distance (δ) and higher values of N, including up to the largest possible value of N, mean more covering markers. And as stated above, thousands, even millions of such bi-allelic covering markers were estimated to be available for future use in linkage studies (and related techniques).

Support for the limitation in new claim 179. This claim contains the limitation *"wherein the species is not human."* The application teaches a wide range of species of creatures, both plant and animal. Human species are specifically taught, see '718 app. p. 12 lines 1-6, and for example, p. 29 lines 13-14, PCT p. 11 lines 1-6, and for example, p. 28 lines 4-5 and Prov. '102 p. 25 lines 1-7 and for example, p. 40 lines 15-16.

Since the application teaches human species, it also teaches species that are not human, see MPEP 2173.05(i) Negative Limitations: *"If alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. In re Johnson, 558 F.2d 1008, 1019, 194 USPQ 187, 196 (CCPA 1977) ('[the] specification, having described the whole, necessarily described the part remaining.')."*

Support for the limitation(s) in new claim 180. This claim contains two true limitations *"wherein the species is animal"* and *"wherein each oligonucleotide in the set is not a 15 nucleotide oligomer."* (The whereby clause is not a true limitation.) Plant and animal species are taught in '718 app. p. 12 lines 1-2, PCT p. 11 lines 1-2 and Prov. '102 p. 25 lines 1-2.

The application teaches complementary oligonucleotides wherein each oligonucleotide

is a 15-nucleotide oligonucleotide. Specifically complementary oligonucleotides in oligonucleotide arrays that are *"15-nucleotide oligomers"* are taught, see bottom right column of p. 610 of the Chee reference, especially the last sentence on p. 610. The Chee reference is incorporated by reference into the application, see '718 app. p. 35 lines 10-11, and p. 49 line 24 and endnote 8, PCT p. 34 lines 5-6, and p. 48 lines 11-12 and endnote VIII, Prov. '102 and p. 53 lines 39-46, p. 125 and endnote i, lines 1-2. **The Chee reference is listed as reference D1 in the Information Disclosure Statement of 5/19/2008 and a copy of the Chee reference was provided to the Examiner.**

The application thus teaches complementary oligonucleotides wherein each oligonucleotide is <u>not</u> a 15-nucleotide oligonucleotide. See MPEP 2173.05(i) <u>Negative Limitations:</u> *"If alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. In re Johnson, 558 F.2d 1008, 1019, 194 USPQ 187, 196 (CCPA 1977) ('[the] specification, having described the whole, necessarily described the part remaining.')."*

Support for the limitation(s) in new claim 181. This claim is identical to claim 180, except "plant" has been substituted for "animal" in this claim. Support for the two true limitations in claim 181 is discussed above under claim 180. The application teaches both "plants and animals."

Support for the limitation(s) in new claim 182. This claim is very similar to claim 170, except that the values for "x" and "y" are stated in terms of approximate values, i.e. "about," rather than in terms of exact values as in claim 170. Support for stating "x" and "y" in terms of such approximate values is given at '718 app. p. 27 lines 29-31, PCT p. 26 lines 20-22 and Prov. '102 p. 35 lines 21-22. Support for the limitation *"N is greater than 2"* was discussed above in Remarks under support of claim 168. The whereby clause in claim 182 is almost identical to the whereby clause in claim 170, except that *"less than or equal to 0.1"* in claim 170 has been replaced by *"less than or*

equal to about 0.1" in claim 182, reflecting the fact that "y" is stated in terms of an approximate value.

Support for the limitation(s) in new claim 183. See Remarks under support for the limitations in claims 171-178.

Support for the limitation in new claim 184. See above under support for new claim 180.

Support for the limitation in new claim 185. Independent claim 91 contains an important limitation that the length of the segment of the segment-subrange (the CL-F region) is *"greater than or equal to the length of human chromosome 21,"* the shortest human chromosome about 47 million bp long. New dependent claims 185 contains a limitation that the length of this segment *"is greater than or equal to the length of human chromosome number 6"* (about 171 million bp long). (The applicants will supply the Examiner with information from NIH and the National Library of Medicine (published Feb. 17, 2009) indicating the length of human chromosome 6 is about 171 million base pairs long.)

The support for this new limitation is similar to that for the similar limitation in independent claim 91 and is found in the same parts of the application(s). Specifically the application, (and PCT parent and Provisional priority application) describe the segment of a segment-subrange as any length up to the length of an entire chromosome. And individual human chromosomes 1-22, X and Y are described. **An example of the chromosomal location coordinates of CL-F points ranging over an entire chromosome, e.g. chromosome number 6, is given.** Frequency subranges and chromosomal segments are described. See for example, '718 app. p. 14 lines 2-6, lines 10-13, p. 38 lines 29-30, p. 44 lines 26-27; PCT p. 13 lines 2-6, lines 10-13, p. 37 lines 15-16, p. 43 lines 26-27 and Prov. '102 p. 30 lines 27-30, lines 38-39, p. 40 lines 44-48, and p. 72 lines 37-38.

Each of chromosomes human chromosomes 1-22, and X and Y, including human chromosome 21, is thus an example of a described possible segment (and segment length) of a segment-subrange. As stated above, the segment of a segment-subrange

is described as any length up to the length of any chromosome. **And the example length of human chromosome 6 then acts as a described "range endpoint" for segment length as in the case In re Wertheim.** See for example, MPEP 2163.05 III. Range Limitations *"In the decision in In re Wertheim, 541 F.2d 257, 191 USPQ 90 (CCPA 1976), the ranges described in the original specification included a range of '25%- 60%' and specific examples of '36%' and '50%.' A corresponding new claim limitation to "at least 35%" did not meet the description requirement because the phrase "at least" had no upper limit and caused the claim to read literally on embodiments outside the "25% to 60%" range, however a limitation to 'between 35% and 60%' did meet the description requirement."*

Regarding the "whereby clause" in new claim 185. This whereby clause is not a true limitation. The true limitations in new claim 185 mean that the minimum number of covering markers in the "whereby clause" for claim 185 is increased by a factor of about 3.6 (i.e., 3.6 ≈ 171 bp/47 bp) over the corresponding figure in claim 170, the claim from which claim 185 depends. The minimum number of covering markers in the "whereby clause" for claim 170, 288, is multiplied by 3.6 to yield *"about 1037"* for new claim 185. That is, the minimum number of covering markers in the "whereby clause" for claim 185 is *"about 1037."*

The other parameters for the covering markers in the "whereby clause" for claim 185 (minimum approximate density and maximum least common allele frequency) are unchanged. That is, the values of each of these parameters in the whereby clause for new claim 185 is the same value as in claim 170, the claim from which claim 185 depends.

Support for the limitation in new claims 186-195. Each of these claims contains the limitation *"wherein the species is human and the chromosomal location coordinates of CL-F points in the CL-F region range over an entire human chromosome, whereby the length of the segment of the segment-subrange is the length of the entire human chromosome over which the chromosomal location coordinates of CL-F points in the CL-F region range."*

The application, (and PCT parent and Provisional priority application) describe the segment of a segment-subrange as any length up to the length of an entire chromosome. And individual human chromosomes 1-22, X and Y are described. An example of the chromosomal location coordinates of CL-F points ranging over an entire chromosome, e.g. chromosome number 6, is given. Frequency subranges and chromosomal segments are described. See for example, '718 app. p. 14 lines 2-6, lines 10-13, p. 38 lines 29-30, p. 44 lines 26-27; PCT p. 13 lines 2-6, lines 10-13, p. 37 lines 15-16, p. 43 lines 26-27 and Prov. '102 p. 30 lines 27-30, lines 38-39, p. 40 lines 44-48, and p. 72 lines 37-38.

Support for the limitations in new claims 196-199. Each of these claims contains one of two limitations *"wherein each oligonucleotide in the set is* (or *is not*) *a 15 nucleotide oligomer."* As stated above under support for claim 180, each of these two limitations is supported, see above Remarks under support for claim 180.

Support for the limitation in new claim 200. This claim contains the limitation *"wherein the chosen group of covering markers including thousands of bi-allelic markers."* Support for this limitation is discussed above in the Remarks under support for claims 171-178.

Support for the limitations in new claims 201-211. The limitations in these claims refer to *"a high-density oligonucleotide array," "a glass slide"* and *"a silicon chip."* These limitations are identical to limitations in previous claims 111-123 of the previous claim set of May 1, 2008; and these limitations are therefore not new. Support for these limitations was discussed previously in the Preliminary Amendment of October 2, 2007 (see p. 36). For the Examiner's convenience the applicants will now cite support for these limitations again. See '718 app. p. 35 lines 10-11; PCT p. 34 lines 5-6 and Prov. '102 p. 53 lines 42-43. Each of these sections refers to the Chee reference (Accessing Genetic Information with High-Density DNA Arrays, Mark Chee, et al.

Science, vol. 274, Oct. 25, 1996, pp. 610 – 614) which is incorporated by reference into ‘718 app., PCT and Prov. ‘102. See Remarks above under support for claim 180 for more details regarding the Chee reference.

Support for the limitations in new dependent claim 212. For support for the limitations in this claim, see for example, ‘718 app. p. 22 lines 10-16, especially lines 14-16, p. 35 lines 10-11 and 13-15; PCT p. 21 lines 1-7, especially lines 5-7, p. 34 lines 5-6 and 8-10 and Prov. ‘102 p. 28 lines 23-28, especially lines 26-28, p. 53 line 39 to p. 54 line 22, especially p. 54 lines 4-22. These passages describe complementary oligonucleotides as PCR primers, signal generation by PCR reactions and a signal such as a dye color.

The Chee reference is also part of these passages. The Abstract of the Chee reference states: *“Sequence polymorphisms were detected with single base resolution…”* The Chee reference is incorporated by reference into ‘718 app., PCT and Prov. ‘102. See Remarks above under support for claim 180 for more details regarding the Chee reference.

Support for the limitations in new claims 213-235.

Support for each of the limitations in new claims 213-235 has already been cited above in Remarks regarding support for specific claims, except for the limitations in claims 224-233 that deal with signals and specific dyes. Support for the limitations in claims 224-233 that deal with signals and specific dyes will be cited when these claims are discussed below, along with support for other claims, in order of claim number and in their turn. It should be noted, however, that the passages in ‘718 app., PCT and Prov. ‘102 cited just above under support for claim 212 support limitations that deal with signals. Additionally, these passages also support PCR primers labeled with specific dyes.

Support for PCR primers labeled with specific dyes. The passages in ‘718 app., PCT and Prov. ‘102 cited just above under support for claim 212 also refer to the

Schuster reference, see ‘718 app. p. 35 lines 13-15 and endnote 10 p. 50, PCT p. 34 lines 8-10 and endnote X p. 48 and Prov. ‘102 p. 54 lines 21-22 and endnote iii p. 125. **PCR primers labeled with specific dyes are described in the Schuster reference:** *“Forward primers are labeled with either 6-FAM, HEX or TET fluorescent dyes…,”* see p. 100 bottom left column. The Schuster reference is Schuster, H. et al (1995) Nature Genetics, 13(1): 98 – 100. The Schuster reference is incorporated by reference into ‘718 app., PCT and Prov. ‘102, see ‘718 app. p. 49 line 24, PCT p. 48 lines 11-2, and Prov. ‘102 p. 125 endnote iii. A copy of p. 100 of the Schuster reference has been previously supplied, see Image File Wrapper entry (IFW) dated 7-16-2006 Applicant Arguments/Remarks (REM) for the present application, last page (page 6). (The journal citation (nature genetics volume 13 1996) and page number (100) are at the bottom of this IFW page copy.)

Support for the limitation in new claim 213. See Remarks above under support for claims 186-195.

Support for the limitation in new claim 214. See Remarks above under support for claims 171-178.

Support for the limitations in new claim 215. See Remarks above under support for claim 168.

Support for the limitation in new claim 216. See Remarks above under support for claims 186-195.

Support for the limitation in new claims 217-218. See Remarks above under support for claims 171-178.

Support for the limitations in new claim 219. See Remarks above under support for claim 170.

Support for the limitation in new claim 220. See Remarks above under support for claims 186-195.

Support for the limitation in new claims 221-222. See Remarks above under support for claims 171-178.

Support for the limitation in new claim 223. See Remarks above under support for claim 179.

Support for the limitation in new claim 224, *"the species is a plant species,"* see Remarks above under support for claim 181.

Support for limitations in new claim 224-233. The remaining limitations in claims 224-233 deal with signals and specific dyes. Each of these claims depends from claim 212. As stated above in the Remarks under support for claim 212, these limitations that deal with signals and specific dyes are supported. And specific passages in '718 app., PCT and Prov. '102 are cited. More specifically support for PCR primers labeled with specific dyes (e.g., TET and HEX) is in the Schuster reference, which is part of the present application (and PCT parent and priority Prov. '102) through incorporation by reference.

The limitation in new claims 231-233 is *"wherein the signal does not include a dye color."* Specific passages in the present application (and PCT & Prov. '102) teach a general signal (physico-chemical signal) and a specific example of a signal as a dye color, see '718 app. p. 22 lines 10-16, PCT p. 21 lines 1-7 and Prov. '102 p. 28 lines 23-28. Therefore the the present application (and PCT & Prov. '102) teach the limitation *"wherein the signal does not include a dye color."* See MPEP 2173.05(i) <u>Negative Limitations:</u> *"If alternative elements are positively recited in the specification, they may be explicitly excluded in the claims. In re Johnson, 558 F.2d 1008, 1019, 194 USPQ 187, 196 (CCPA 1977) ('[the] specification, having described the whole, necessarily described the part remaining.')."*

Support for limitations in new claim 234. See Remarks above under support for claim 185.

Support for limitations in new claim 235. See Remarks above under support for claim 182.

Some further Remarks regarding interpretation of the claims and claim terminology

The applicants now make some further Remarks regarding interpretation of the claims and claim terminology.

Independent claim 91, and all the other pending claims, which depend from claim 91, contain act or step type process limitations. Specifically these are *"wherein the set of oligonucleotides is selected for the set's utility to determine genotype data or sample allele frequency data for each of the two or more covering markers"* and *"wherein the group of covering markers is chosen so that a CL-F region is N-covered to within [x, y] by the covering markers."*

Patentability of a product defined by process limitations is generally determined by the product. *"[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself."* In re Thorpe, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985) (quoted from MPEP 2113). The applicants now state, however, that for each of the claims in the presently pending claim set <u>to be infringed</u>, the process type limitations above (or their equivalents) must be infringed. Such a claim construction is a possibility. As the Court in Atlantic Thermoplastics Co. vs. Faytex Corp. (970 F. 2d 834, 23 USPQ2d 1481; Fed. Cir. 1992) stated: *" 'A "product by process" claim is one in which the product is defined at least in part in terms of the method or process by which it is made. Most decisions hold that such a claim is infringed only by a product made through a substantially identical process…' "* (quoting 2 DONALD CHISUM, PATENTS §8.05 (1991)). (In making this statement about infringement the applicants are not, however, forfeiting the future right (in <u>other claims</u> in the present or child applications) to claim a product (or products) defined by the presently pending claims that is (are) <u>not made</u> by practicing the process type limitations (or equivalents) in claim 91.)

Further Remarks regarding claim construction. A product that (1) is made using bi-allelic markers that were identified at the time of filing of the Provisional priority application (60/076102, filed 26 Feb 1998), or (2) is made using bi-allelic markers that were not yet identified at the time of filing of 60/076102, or a combination of (1) and (2) that meets the limitations of any one of the pending claims is within the scope of the claim. At the time of filing it was known in the art that thousands, even millions, of bi-allelic markers (SNPs) would likely become identified and become available for future use in linkage studies.

Most of the bi-allelic markers now presently available were not identified by 26 Feb 1998. See for example p. 21 mid right column of Kruglyak (The use of a genetic map of biallelic markers in linkage studies. Nature Genetics, September 1997, vol.17, pp. 21-24) that states *"..classic estimates of more than 1 per 1,000 base pairs, or more than 3 million* [SNPs] *in the genome. To date, more than 1,000 PCR amplifiable SNP markers have been discovered and mapped (D. Wang, pers. comm.)."* It should be noted that 3 million SNPs in the entire genome translates to about 130, 000 SNPs (3 million/23) per human chromosome. (More details on the Kruglyak (1997) reference are given above on p. 22.)

Significant efforts were, however, underway by about 26 Feb 1998 to identify SNPs for future use. For example, see Abstract of a slide presentation: Wang, D. et al. Toward a third generation genetic map of the human genome based on biallelic polymorphisms. Am. J. Hum. Genet. 59, A3 (1996). The Abstract states that 400 SNPs had been discovered by the authors. This Abstract, like other conventional art cited above, describes SNPs with minor allele frequencies > 30%. See also Kwok, et. al., (Genome Res. 1998 8: 748-754, Overlapping Genomic Sequences: A Treasure Trove of Single-Nucleotide Polymorphisms), this paper describes finding 153 SNPs over a 200.6 kb region. A paper by Kwok, et. al. (Hum Mutat. 1998;12(4):221-5 Single nucleotide polymorphism hunting in cyberspace) describes finding SNPs by computer analysis of data. A Wang reference (Large Scale Identification, Mapping, and Genotyping of Single-Nucleotide Polymorphisms in the Human Genome, Wang, et. al., Science, May

15, 1998, vol 280, pp. 1077-1081) describes finding 3241 candidate SNPs and mapping 2227 of these SNPs (a 500 SNP genotyping chip is also described). This Wang reference is referred to in the present application on p. 35 lines 11-13 and endnote 9 p. 49. A copy of the Wang slide presentation Abstract, both Kwok papers and this Wang reference will be supplied to the Examiner for his convenience.

The covering distances [x, y] in the pending claims are based on subject matter common to each of the present application, the PCT parent and priority Provisional (‘718 app., PCT, and Prov. ‘102). This common subject matter is included in each of ‘718 app., PCT, and Prov. ‘102 and describes “increased power” when linkage disequilibrium is present, wherein the linkage disequilibrium is “positive linkage disequilibrium.” Positive linkage disequilibrium is described in Theory of Operation Section(s) of in each of ‘718 app., PCT, and Prov. ‘102. “Positive linkage disequilibrium” is present when two bi-allelic polymorphisms are linked and the minor (and major) alleles of the two polymorphisms are positively associated. Positive linkage disequilibrium is also illustrated by examples in Table 2 in each of ‘718 app., PCT, and Prov. ‘102. See for example ‘718 app., p. 45 lines 5-8 and p. 46 lines 29-31, PCT p. 44 lines 5-8 and p. 45 lines 29-31, and Prov. ‘102 p. 39 lines 40-42, p. 82 lines 8-11. Even though the covering distances are based on the above criteria defined by positive linkage disequilibrum, it is possible, however, for one or more chosen covering markers to be in linkage disequilibrium (or exhibit linkage disequilibrium to) a sought trait-causing polymorphism (in the CL-F region), wherein the linkage disequilibrium is not “positive linkage disequilibrium.”

Common subject matter (included in each of ‘718 app., PCT, and Prov. ‘102) also includes information in AHG98 (page 164) that describes the use of sample size n_{tdt} and binomial probability P_t to generate a binomial distribution. And as AHG98 states, standard tables giving the normal approximation to the binomial distribution (Pearson & Hartley, 1954; Weir 1996) provide precise power values for virtually any sample size (n_{tdt}), binomial probability (P_t) and significance level. This information is also present in

the inventor's unpublished manuscript (entitled Detection of linkage: Comparison of the affected sib pair (ASP) test and transmission/disequilibrium test (TDT). As noted above, AHG98 is incorporated by reference into '718 app. & PCT and the unpublished manuscript incorporated by reference into Prov. '102. A full copy of the inventor's unpublished manuscript was included with, and incorporated by reference into, Provisional priority application 60/076182, filed 27Feb1998 (page 28 was missing from the copy of the inventor's unpublished manuscript included with Prov. '102).

Just as a subset can contain every member of a set, it is possible for the subrange of a segment-subrange to be the minor allele frequency subrange (or range) "0 to 0.5." This is indicated, for example, by claim 104 in the presently pending set of claims. As further (intrinsic) evidence of this interpretation, see for example, p. 26 lines 19-21 of Provisional priority application 60/076102 (Prov. '102), that states: *"The **width of a subrange** is the absolute difference between the subrange's upper limit and it's lower limit. For example, for the subrange 0.1 to 0.3, the width of the subrange is 0.2, i.e. 0.3 – 0.1 = 0.2. The subrange with the greatest width is the range 0 to 0.5. The greatest subrange width is 0.5"*

Conclusion

The applicants have responded to rejections in the non-final Office Action of 9/22/2008 by amending claims 91-93, 104-105, 108 and 109, and canceling claims 94-103, 106-107, and 110-166. New claims 167-235 were added for a total of 76 pending claims, unchanged from the previous total. A small entity three-month extension fee under 37 CFR 1.136(a) will be paid separately.

The applicants have addressed rejections regarding a lack of priority and indefiniteness related to the claim limitation “thousands of bi-allelic markers.” And several “whereby clauses” that are not true limitations were included in many claims to help to more clearly delineate the “metes and bounds” of the claimed invention(s).

Remarks regarding the proper construction of claims that include the limitation “thousands of bi-allelic markers” were made and support was cited to eliminate later references McGinnis (1999) and Cohen (1999) as “prior art” against the pending claims. A great deal of objective evidence of the achievement of “unexpected results” by the claimed invention(s) was presented. A rebuttal of the case of prima facie obviousness based on the Cohen (1997) and Kruglyak (1995) references was made; these references lead away from the claimed invention(s).

Support for limitations in the presently pending claims to ensure compliance with the Written Description Requirement of 35 U.S.C. 112 is cited in the text of the present application, the PCT parent and a Provisional priority application was cited. This support ensures that later filed references (such as McGinnis (1999) and Cohen (1999)) are not “prior art” against the presently pending claims. Some further Remarks regarding interpretation of the claims, claim terminology, and claim construction including claim construction of product by process type claims were also made.

For the reasons advanced above, applicants respectfully submit that the claims are now in condition for allowance and that action is earnestly solicited.

Respectfully submitted,

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Attached:

1) Exhibit A: Rejection letter from Peter Byers, editor of the American Journal of Human Genetics, dated January 28, 1997 with Reviewer Comments from Reviewers A and B, total of 4 pages.

Supporting documents sent separately:

2) Genetics Home Reference information from NIH and the National Library of Medicine on the lengths of Human Chromosome Number 6 (4 pages published Feb. 17, 2009) and Human Chromosome Number 21 (1 page reviewed June 2008).

3) Abstract of journal article Reich, et. al (Nature. 2001 May 10;411(6834):199-204.) and three journal articles: Wang, et. al. (Genetics and molecular research 2007 Dec 11;6(4):1131-41. Kolkman, et. al, (Genetics. 2007 Sep;177(1):457-68.) Du, et. al., (Int J Biol Sci. 2007 Feb 10;3(3):166-78.)

4) Slide presentation Abstract (Wang, D. et al. Am. J. Hum. Genet. 59, A3 (1996), 1 page. Three published papers: Kwok, et. al. (Hum Mutat. 1998;12(4):221-5); Kwok, et. al., (Genome Res. 1998 8: 748-754). Wang, et. al. (Science, May 15, 1998, vol 280, pp. 1077-1082)

<u>Exhibit A</u>

Exhibit A is a rejection letter from Peter Byers, editor of the American Journal of Human Genetics, dated January 28, 1997 with associated Academic Reviewer Comments from Reviewers A and B, a total of 4 pages. Exhibit A is cited as evidence of unobviousness in this Amendment/Response for Application No. 10/037, 718, see pp. 34-36.

As stated in the Remarks Section of this Amendment/Response (p. 36) *"The identity of these Reviewers is unknown to the applicants. But the applicants respectfully submit that, as is customary, the academic Reviewers in this case at the <u>American Journal of Human Genetics</u> were high-quality conscientious Reviewers.* ***And the applicants respectfully submit that the reason the unpublished manuscript was <u>initially rejected</u> for publication is because the results in the unpublished manuscript, though correct, were so contrary to conventional thinking in the art of linkage studies at the time of review and <u>were unobvious</u>."***

<u>Exhibit A is attached to this paper as the next four pages</u>

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January 28, 1997

Dr. Ralph E. McGinnis
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415 Curie Blvd, 470 CRB
Philadelphia, PA 19104-6145

Dear Dr. McGinnis:

Thank you for submitting your manuscript, "Detection of Linkage: Comparison of the Affected Sib Pair (ASP) Test and Transmission/Disequilibrium Test (TDT)," to *The American Journal of Human Genetics*. Your paper has been evaluated by two outside reviewers. I regret to say that we are unable to accept it for publication. The reviewers did not assign the paper a high enough priority to warrant publication in the *Journal*.

Attached are the reviewers' comments. We hope they will be helpful to you. We are returning your manuscript under separate cover.

Thank you for the opportunity to consider your work.

Sincerely yours,

Peter H. Byers

Peter H. Byers, MD
Editor

MS AJHGA960767

Manuscript no. AJHG[illegible] Reviewer A B C

The American Journal of Human Genetics

Manuscript Evaluation Form

COMMENTS TO AUTHOR

Reviewer: Please *do not* indicate to the author your recommendation regarding publication of the paper. Please FAX this review to *AJHG* (206)685-8684. Thank you.

Author: Ralph E. McGinnis
Title: Comparison of TDT and ASP Tests

Linkage analysis may be used to detect genetic risk factors involved in multifactorial diseases and the screening of the whole genome by the ASP method has become very fashionable. In the situation where the genetic marker is close enough to the disease predisposing factor, there may be allelic association between the marker and the risk factor alleles which may increase the power to detect the effect of the last one. Then, the TDT is a very interesting method using both association and linkage information. The aim of the study, which is to examine the comparative powers of the two approaches ASP and TDT, is thus very important.

However, I think that the message given in the present study is a little misleading in applying both the ASP and TDT method to a biallelic marker. Because the allele sharing in sib pairs is informative only when parents are heterozygous, the ASP method is generally applied to situations where the marker (or the set of closely linked markers) is very informative (with an heterozygosity rate close to 1). In this more realistic situation, the results of comparative power will not be exactly the same (see the study of Clerget-Darpoux et al, Genet Epidemiol, 1995).

Even if unrealistic, there is a clear advantage of choosing the situation of a biallelic marker. This advantage is the possibility to consider for each method a binomial distribution with respective probability Ps and Pt which are expressed in such a way that it is possible to deal with the numerous parameters influencing the power of these methods. In particular, it is very nice to see how the ratio r of the penetrances of the two homozygotes DD and dd affect the power of each test. However, I have two remarks :

- The observation that the ratio Rt/Rs increases to ∞ when r goes to 1 should be moderated by the fact that the two terms Rt and Rs decrease to 0
- Besides the effect of r, it seems to me important to discuss the one of x. The power of ASP varies a lot with x. In particular for a frequent disease allele and under the assumption of no phenocopy, the power of ASP is good for x close to 0 but decreases very quickly when x increases.

Once again, my only but major concern is to take a biallelic marker for applying ASP, and I do not know if the author may reconsider this assumption while keeping the same smart reasonning. Note also that it makes irrelevant all the discussion about choosing a marker with an heterozygozity rate equal to the one of the disease locus (which in any case is unknown).

Typo error in equation 2 p 7 (c1c4-c2c3) instead of (c1c4-c2c4).

Please attach additional pages if necessary.

Manuscript no. AJHGA960767 Reviewer A B C

The American Journal of Human Genetics

Manuscript Evaluation Form

COMMENTS TO AUTHOR

Reviewer: Please *do not* indicate to the author your recommendation regarding publication of the paper. Please FAX this review to *AJHG* (206)685-8684. Thank you.

Author: Ralph E. McGinnis
Title: Comparison of TDT and ASP Tests

The author compares the power to detect linkage by the TDT and ASP test in case of a general single locus disease model. He argues that this comparison can be reduced to compare two binomial probabilities P_t and P_s, which are functions of the haplotype frequencies, penetrances and recombination fraction. I have several problems with this approach:

1. The natural basis for comparing the TDT and ASP test is a sample of n affected sib-pairs. While the ASP test can use all these families (given that the marker is polymorphic, so that the parents are informative), the TDT can use only a fraction of the families/parents. The author starts his comparison by assuming a sample of affected sib-pairs with an A/B heterozygous parent. This is unrealistic (nobody can select with regard to the marker genotype of the parents) and handicaps the ASP test. Although the author presents formulas for correcting this unrealistic assumption, most of the conclusions are drawn simply on the basis of P_t and P_s.

2. On page 34, the author correctly states that the power of the TDT can only approximately be calculated from P_t. He claims that this approximation is quite precise. This may be true; however, I don't understand his argument(being presented on page 35) to support this claim. Similary, it should be noted that no single parameter like P_s is sufficient to calculate the power of the ASP test in case that the sib-pairs are not selected with regard to the parental marker genotype.

3. A further argument which shows that the comparison of P_t and P_s is not suited to compare the power of the TDT and ASP test is that the mean test should be performed as a one-sided test (see Blackwelder and Elston, 1985). Thus, even if one would be willing to ignore 1. and 2., the

Please attach additional pages if necessary.

power of the ASP test can still be larger than the power of the TDT, even in situations where $P_s < P_t$.

The paper contains a lot of algebra and I must admit that it was impossible for me to check every detail. I am quite convinced that the basic equations 1 and 2 on page 7 are correct (irrespective of an obvious typo in equation 2: it should read $-c_3 \cdot c_5$ instead of $-c_3 \cdot c_4$) for heterozygous A/B parents *in families with exactly two affected children*. I seriously doubt that the same expressions should be valid, irrespective of the family size and the number of affecteds, as pretended on page 28. A simple argument which shows that equation 1 is not correct for A/B parents of k affected offsprings and sibship size k is as follows: Suppose a recessive disease and no phaenocopies (i.e., $\alpha = 1$, $\beta = \gamma = 0$). Further, assume there is no linkage disequilibrium and $\theta = 0$. Then, for $k \to \infty$, the proportion of parental mating type $DD \times DD$ in these families converges to 1. Therefore, $P_s \to 1/2$. The error in appendix A seems to be in the second column of table 4. On page 29, it is explained that the last factor in this column is "the conditional probability that the mating type produces at least k affected offspring from exactly N children", and in the first row of table 4, this factor becomes $\binom{N}{k}((\alpha+\beta)/2)^k$. But the correct probability for the mating type $Dd \times DD$ to produce at least k affected offspring from exactly N children is $\sum_{j \geq k}^{N} \binom{N}{j}((\alpha+\beta)/2)^j(1-(\alpha+\beta)/2)^{N-j}$.

I also doubt that what the author calls "perhaps the most important finding of this paper; namely the importance of using bi-allelic markers with heterozygosity similar to that of a bi-allelic disease locus"(page 25) is a advice really justified. This result is based on comparing the power of the TDT for different marker allele frequencies and $\delta = \delta_{max}$, $\delta = \delta_{max}/2$ (Table 2). The author does not take into account that the situation of a rare marker allele being in positive linkage disequilibrium with disease allele D may be quite infrequent. For example, consider a simplistic model where all disease carrying haplotypes are copies of the same ancestral mutated haplotype. It is unlikely that the original mutation occured on a haplotype carrying the rare marker allele.

Finally, I dislike the statement "Linkage disequilibrium between marker and disease locus increases power to detect linkage ...for the ASP test" (Summary and page 3). The author knows that this statement is simply wrong without the artifical assumption of selecting affected sib-pairs according to their parental marker genotype and may be very confusing for those not reading the entire manuscript carefully.